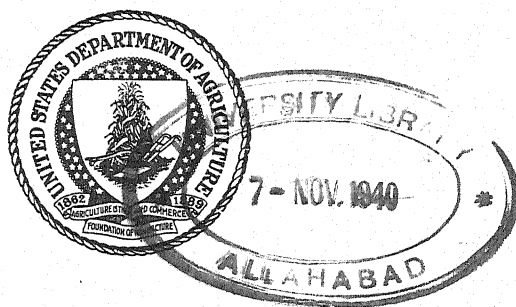


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AUTHOR'S ERRATA AND EMENDATION

- Page 67, line 6, "532.94" should be "332.94."
- Page 67, table 3, column 4, line 1, "407.20" should be "4076.20."
- Page 68, line 15, " $(\bar{x}' - \bar{x}'')$ 0.05" should be " $(\bar{x}' - \bar{x}'')$ 0.05," and " $(\bar{x}' - \bar{x}'')$ 0.01" should be " $(\bar{x}' - \bar{x}'')$ 0.01."
- Page 68, footnote 6, " $N_2=42$ " should be " $N_1=42$."
- Page 76, under heading, "Experiments with Other Aphids," lines 3 and 4, delete the words, "all of which were grown from seed."
- Page 101, seventh line from bottom, "varieties of" should be "varieties or."
- Page 105, tenth line from bottom, "north and south" should be "east and west."
- Page 106, line 9, "longitudinal" should be "crosswise."
- Page 112, line 11, " $-3Z_{..w}$ " should be " $+3Z_{..w}$."
- Page 116, lines 19 and 20, " $n=16$ ($n=n_1+n_2$ where n_1+1 and n_2+1 are each equal to 9)" should be " $n=5,104$ the number of degrees of freedom available for the estimation of error."
- Page 117, line 17, " $\frac{\Sigma(X_{..w}) + \Sigma(Y_{..w}) + \Sigma(Z_{..w})}{27}$ " should be " $\frac{\Sigma(X_{..w})^2 + \Sigma(Y_{..w})^2 + \Sigma(Z_{..w})^2}{27}$."
- Page 136, line 11, insert the word "regain" before "its."
- Page 148, table 6, first word of heading "Sucrose," should be "Reducing sugars."
- Page 203, table 5, headings, "Sum of square" should be "Sum of squares."
- Page 385, first line, "S. Watts" should be "S. Wats."
- Page 402, figure 3, magnifications should read A, B, $\times 65$; C $\times 60$; D, E, $\times 1$.
- Page 481, table 5, column 1, "I" should be "IX."
- Page 511, table 3, delete superscripts "3" from all data applying to leachings; for HPO_3S_1 middle pot, change superscript "1" to "2" for 1.78; for NaH_2PO_2 c. p., 4₁ middle pot, change superscript "2" to "3" for .66; for $Na_4P_2O_7$, c. p., 4₁ and $C_6(H_2PO_4)_2$ c. p., 4₁ top pot, change all six of superscripts from "1" to "2."
- Page 595, figure 2, "32°" should be "30°."
- Page 598, table 5, last column, the following figures should appear, from top to bottom: 54, 38, 42, 46, 75, 71, 43, 39, 36, 35, 68, and 75.
- Page 657, legend for figure 9, "perfect" should be "imperfect."
- Page 674, table 3, note under heading, "h=100 except where noted" should be "n=100 except where noted."
- Page 679, figure 1, on both abscissa and ordinate "0.1" should be "0.01."
- Page 693, table 5, the units for the last six columns from left to right are: "Percent, Percent, Number, Number, Percent, and Percent."
- Page 743, line 18, "whereas with nitrate" should be "whereas with nitrite."
- Page 760, line 15 of text, "its action on the fungus" should be "scandium's action on the fungus."
- Page 777, ninth line from bottom, "5.66 for fat and 9.35 for protein" should be "9.35 for fat and 5.66 for protein."
- Page 809, figure 1, last line of legend, "a and b more than strong plants c and d" should be "plant b more than strong plant d."
- Page 859, table 1, next to last column, line 15, "1909-1934" for Fort Valley should be "1909-1930."

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No. 1

INFLUENCE OF MOISTURE SUPPLY ON DROUGHT RESISTANCE OF CONIFERS¹

By HARDY L. SHIRLEY, *senior silviculturist*, and LLOYD J. MEULI, *formerly junior plant physiologist, Lake States Forest Experiment Station, Forest Service, United States Department of Agriculture*²

INTRODUCTION

In many regions of the United States, the success of forest plantings depends to a considerable extent upon the use of well-hardened, drought-resistant planting stock. Over extensive areas of the Western States, the Plains region, and the Lake States severe droughts are common, and only the more drought-resistant species and individuals can be depended upon to survive and make satisfactory growth. Even in regions normally well supplied with rainfall, hardy planting stock is required on the thin or badly eroded soils which are being planted to forest trees.

In northern Minnesota, the unusually dry summer of 1930 caused widespread damage to forest trees; in unfavorable locations, young trees 6 to 8 feet in height were killed (14).³ Severe drought recurred in 1933, 1934, and 1936. Mortality in recently established forest plantations was high in all 4 years. The greatest damage occurred in 1936, when 50 percent of the seedlings newly planted on sandy soils were killed. The drought of 1936 was accompanied by a period of extremely high temperatures, which not only accentuated the moisture stress within the plants but critically injured many seedlings by overheating. Such losses emphasize the need of more exact knowledge of the factors influencing survival of trees during periods of drought, especially factors subject to human control.

Although field observations and biochemical tests have given us a general knowledge of the physiology of drought resistance, exact information on this subject is still signally inadequate. One of the greatest obstacles to experimental investigation of the problem has been the lack of an effective method for evaluating drought resistance. The first step in the study discussed here was to devise an apparatus in which a direct test of drought resistance could be made. By means of the direct test, it is possible to study the influences exerted on drought resistance by external and internal factors. This report presents evidence that the tests made provide a real index of the inherent drought resistance of coniferous seedlings, and evidence as to the influence of previous moisture supply on the drought resistance of the principal conifers of the Lake States. It presents data, also, regarding the persistence of the improved drought resistance built up by treatments applied to seedlings in the nursery.

¹ Received for publication December 7, 1938.

² Acknowledgment is due several other members of the staff of the Lake States Forest Experiment Station who have contributed materially to the study discussed.

³ Italic numbers in parentheses refer to Literature Cited, p. 20.

The field tests described in this paper were made at Cass Lake, on the Chippewa National Forest, Minn., with plants grown in nearby forest nurseries.

REVIEW OF LITERATURE

Both direct and indirect methods have been used to study drought resistance. Studies by indirect methods have contributed much to an understanding of the morphological and physiological responses of plants to drought. However, owing to the extreme complexity of the problem, no simple and clear-cut criteria have been developed whereby the drought resistance of a given plant may be determined with accuracy. Investigators have now begun to adopt direct exposure to drought as the method of determining hardiness. Maximov (10), in an exhaustive study of factors influencing drought resistance, concluded that the capacity to endure prolonged wilting is one of the most important characters influencing drought resistance in plants. Although this conclusion has not yet been verified by extensive experimental evidence, it is significant that students of the closely related problem of cold resistance, having encountered contradictory results from indirect tests (3, 5, 12, 17), now rely almost exclusively upon direct exposure to low temperature to determine winter hardiness.

Marshall (9) studied the reactions of several species of conifer seedlings as they wilted, became desiccated, and died after suspension of watering. Tumanov (18, 19) and other Russian investigators, after subjecting plants to soil dryness, have taken as a criterion of drought resistance the percentage survival or the relative resistance to loss of leaf and stem tissue through desiccation. Krasnosselsky-Maximov (8) subjected plants to hot, dry air in a wind tunnel in order to investigate reactions to drought.

The relative resistance of varieties of maize to combined drought and heat was investigated by Hunter, Laude, and Brunson (6) by use of a closed, heated chamber. As this chamber was operated at 140° F. (60° C.) and at a relative humidity of about 30 percent, the injuries noted may have resulted more from excessive heat than from drought.

For testing the drought resistance of varieties of wheat a more elaborate apparatus was used by Aamodt (1). This consisted of a glass-enclosed tunnel through which air, heated to constant temperature, was blown at a velocity of about 6 miles per hour. A revolving chain belt has recently been installed in this apparatus to insure uniform exposure of the plant material.⁴ In recent work with this apparatus, which is operated in a greenhouse, Peto⁵ has encountered difficulty in comparing the results of replicate tests, owing to lack of control over light intensity.

Tumanov (18) and Kondo (7) have shown that crop plants subjected to periodic wilting while in the growing stage attained greater resistance to subsequent drought than those grown with an abundant moisture supply. Aamodt and Johnston (2) cite several studies confirming this finding and present data of their own indicating that wheat plants can be hardened by subjecting them either to soil dryness or to hot, dry wind. Data presented by Marshall (9) indicate that coniferous seedlings grown in a soil deficient in moisture survived drought longer than those grown in a wet soil.

⁴ According to Prof. K. W. Neatby, of the University of Alberta.

⁵ PETO, HOWARD B. A METHOD OF STUDYING DROUGHT RESISTANCE IN PLANTS. 1937. Unpublished manuscript, Univ. of Alberta.

METHOD EMPLOYED TO TEST DROUGHT RESISTANCE

In preliminary experiments it was found almost impossible to get a reliable test of the relative drought resistance of coniferous seedlings simply by discontinuing the water supply. If the plants remained in the greenhouse, the soil dried unevenly in the different containers. If this difficulty was overcome by adding water, 3 to 6 weeks were required before all plants died. Furthermore light intensity, temperature, relative humidity, and air movement, all of which affect rate of transpiration, were found to be unequal at different locations within the greenhouse. When the same method was applied out of doors, protection from rain was necessary and even then the test required 2 to 4 weeks.

If the influence of various factors on drought resistance is to be studied effectively, not only must all plants in each test lot be uniformly exposed to drought but exposure must be so severe that the results will be free from the influence of secondary hardening. Such hardening was found often to mask the influence of the primary factors in tests of 2 or more weeks' duration. Therefore a procedure was adopted involving continuous exposure to acute desiccation.

DROUGHT MACHINES

The first drought machine used in this study was an improvised apparatus consisting of a large galvanized-iron can inverted over a revolving table on which the potted plants rested. Pans containing calcium chloride were placed under the table, to which fins were attached for fanning air over the desiccant. Tests with this machine, which was constructed in 1932, convinced the senior author of the possibilities of this method. Consequently a carefully planned machine was constructed in 1933. A description of the essential features of this apparatus was published in 1934 (13). With slight modifications, the same equipment was used in a study of lethal high temperatures for coniferous seedlings (15). While satisfactory in other respects, this machine proved to be too small to hold the quantities of experimental material needed when more than two treatments were involved.

In 1936 a third machine (fig. 1) was built on the same principles as the 1933 model. The table, enlarged to 5 feet in diameter, will support as many as 240 individually potted plants in four outer rows where air velocity is approximately constant at 5.2 miles per hour. Instead of a squirrel-cage blower such as was used in 1933, which functions inefficiently in a closed system, a positive-pressure blower, capable of delivering air against a pressure of 3 pounds per square inch, was installed. This consists of two oppositely rotating impellers, mounted on parallel shafts, inside an oval housing. This blower delivers per minute 48 cubic feet of air, almost one-half the total air volume of the machine when filled with plants. Air from the top of the chamber is drawn into a pipe 1½ inches inside diameter and is passed through the pump and into the air drier. This consists of a rectangular box of sheet copper in which 10 pairs of trays holding as much as 15 pounds of calcium chloride can be inserted. The interior is arranged in such a manner that air passing through the drier must flow across the surface of each pair of trays in turn. The dry air is forced vertically down onto the plants as they pass by the door. This breaks up the air mass

immediately surrounding the plants, which otherwise tends to rotate with them. A fan mounted at the ceiling of the chamber has been found useful in further breaking up local eddies and in insuring uniform air temperature.

The sheet-metal drum is made in two vertical halves reinforced with angle iron. All joints are gasketed with $\frac{1}{4}$ -inch sponge rubber, so that the entire system is approximately airtight. The exterior is covered with one to two layers of asbestos paper, to prevent too rapid loss of heat. The interior is coated with a powdered-aluminum paint, which reflects and diffuses light. Heat is supplied by an electric

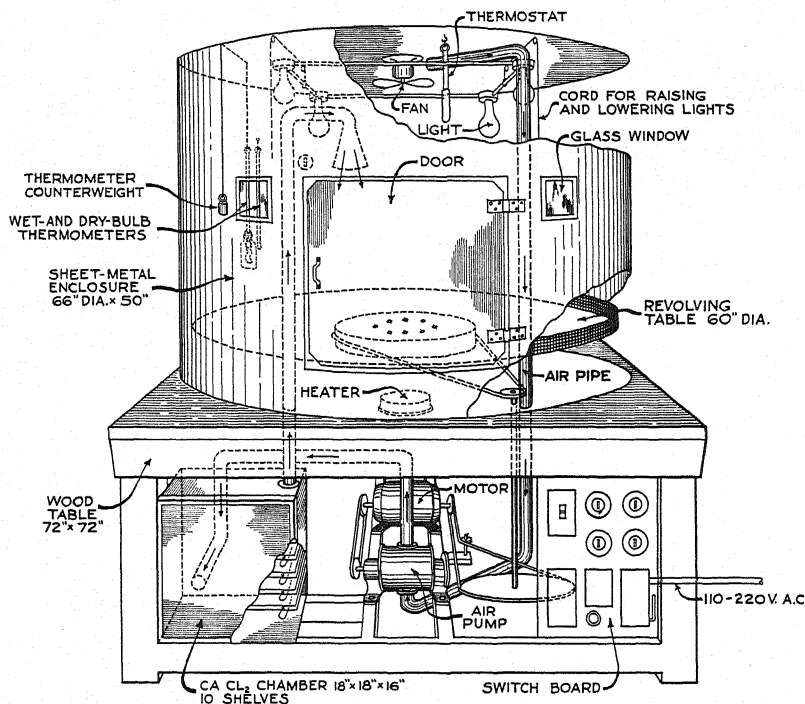


FIGURE 1.—Diagram of 1936 drought machine.

heater mounted under the table and by four lamps totaling 1,000 watts' capacity. These lamps provide, at the level occupied by the plants, a light intensity of 406 foot-candles at a radiation intensity of 0.13 gram-calorie per square centimeter per minute. The heater alone, or the heater and two lamps, can be activated by a mercury thermoregulator sensitive to about 0.1° C. A small low-voltage current through the regulator activates a relay which controls the heater circuit. Both the lamps and the thermoregulator are suspended on springs, which take up vibration. Wet- and dry-bulb thermometers are placed in the stream of outgoing air, which has a current velocity of 443 feet per minute. A special humidifying device (fig. 2) ⁶ delivers steam into the drought chamber when activated by the humidostat.

⁶ This device was designed and constructed by E. E. Aamodt.

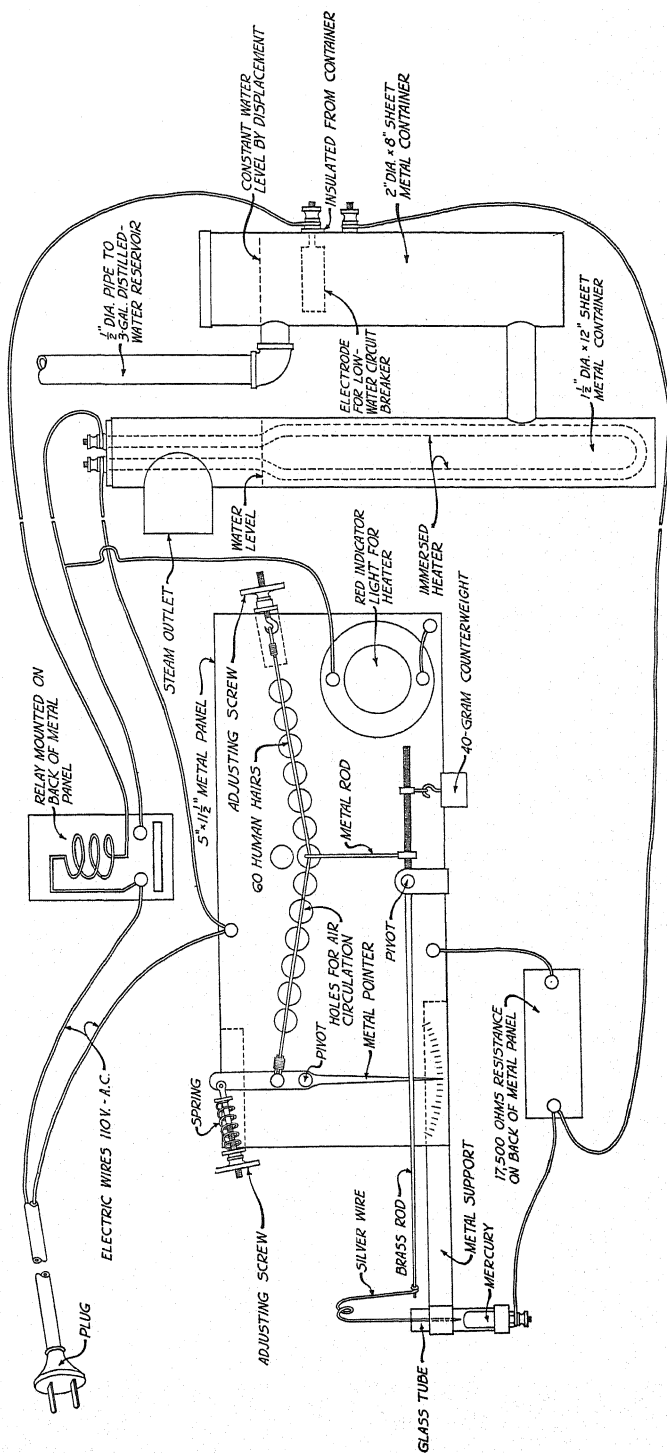


FIGURE 2.—Diagram of humidifier used in 1936 drought machine.

The revolving table is mounted on the front-wheel assembly of a standard motorcar. Even when fully loaded with more than 500 pounds of potted plants the table makes 31 revolutions per minute, with negligible friction. A single $\frac{1}{2}$ -horsepower motor drives both the air pump and the table. All speed reduction is by pulley and belt, which allows flexibility in starting and minimizes friction.

Most tests are run at a temperature of 35° to 37° C., which is well below lethal values for conifers (15). The atmospheric humidity is held up to 20 percent. The accuracy of control possible in this machine, and also in the 1933 machine, is indicated by the temperatures and humidities for 1937 tests (table 1). Since temperature is controlled solely by the addition of heat, it is necessary that the room in which the machine is used remain sufficiently below the operating temperature to dissipate the heat given off by the lamps. The room in which the 1936 machine is operated is maintained at constant temperature by heating and cooling coils activated by thermostats. It has been found that in this room the humidity within the machine can be controlled more easily by venting a small quantity of air than by circulating the air over a desiccant.

TABLE 1.—Temperatures and humidities in drought machines during tests conducted in 1937

1936 machine					1933 machine				
Date of test	Temperature		Relative humidity		Date of test	Temperature		Relative humidity	
	Mean	Standard deviation	Mean	Standard deviation		Mean	Standard deviation	Mean	Standard deviation
	° C.	° C.	Percent	Percent		° C.	° C.	Percent	Percent
May 4.....	36.0	0.4	22	5	July 16.....	37.8	0.5	27	4
May 12.....	36.2	.6	19	3	July 21.....	37.8	.5	24	3
June 3.....	36.1	.2	22	2	July 27.....	37.9	.3	31	7
July 6.....	36.1	.5	21	1	Aug. 4.....	37.9	.7	29	4
July 13.....	36.5	.9	24	3	Aug. 10.....	37.5	.8	27	3
Aug. 2.....	36.1	.6	21	2	Aug. 17.....	37.2	.3	24	2
Aug. 11.....	36.0	.2	21	2	Aug. 24.....	37.6	.3	29	4
Sept. 2.....	35.9	.5	23	3	Sept. 1.....	37.7	.4	26	4
Sept. 9.....	35.7	.4	20	2	Sept. 10.....	37.8	.3	27	5
Oct. 5.....	36.2	.6	21	1					
Oct. 14.....	36.8	.7	21	1					

HANDLING OF PLANT MATERIAL

The species used in the tests reported were red pine (*Pinus resinosa*), northern white pine (*P. strobus*), and jack pine (*P. banksiana*). Stocks of individual species used for comparative tests were in each case derived from the same seed source.

Although other factors could be held essentially uniform, the plant material was likely to vary widely from one test to another. To avoid error in interpretation from this cause, the material used in every individual test included control plants. Experience has shown that 20 plants are sufficient to give a reliable average, hence as many as 12 variations of treatment can be tested simultaneously in the 1936 machine. The fact that coniferous seedlings of the ages used vary appreciably in size made it necessary to arrange the tests in such a way that the effect of size would not obscure the effect of treatment.

This was accomplished by using a factorial design in which size was one of the variables. The data obtained were treated by the analysis-of-variance method (4).

When tests were made on seedlings lifted directly from nursery beds, care was taken to select plants within the same size range to represent each treatment. This was done sometimes by ocular comparison, but more often by weighing each plant. Repeated analyses have shown that minor variations in size of seedlings, such as 1 to 2 grams for 2-0 stock, do not appreciably affect relative drought resistance. On the other hand large variations in size cause very important differences in resistance, the resistance increasing with size.

The seedlings were potted individually in No. 2 or No. 3 tin cans or in 4-inch clay pots. Fine sand having a wilting coefficient of approximately 1.0 percent was used as a potting medium. This was thoroughly mixed and brought to 5-percent moisture content before potting. The plants were placed in the drought machine immediately after potting and were allowed to remain without additional water until all the needles or the stem became brittle, determined by touching or breaking. The plants had then a mean moisture content, based on dry weight, of about 10.5 percent with a standard deviation of 3.1 percent. This end point was chosen as the best practicable criterion of death. The length of time each plant remained alive was used as an index of its drought resistance.

VALIDITY OF MACHINE TESTS

In order that the severity of drought to which plants are exposed in the machine may be appreciated more fully, data are presented in table 2 as to weather conditions at Cass Lake, Minn., during the 1936 drought, a period of 58 days in which precipitation totaled only 1.21 inches. During the latter part of that period the soil moisture on the plot where field tests were conducted was but little above the wilting coefficient. To a depth beyond 6 inches, the sandy soil would flow freely between the fingers. On only 8 of the 58 days did the temperature at 5 p. m. approach that maintained in the machine, but 7 of these days were consecutive. The relative humidity reached low values on only 3 days. The wind velocity in the field, on the other hand, appears to have been somewhat greater than that in the machine. It was measured by an anemometer operating 25 feet above the ground level, where wind velocities are from 50 to 100 percent greater than at the level occupied by seedling conifers. Exposure in the machine to a continuous wind of 5.2 miles per hour, therefore, was probably more severe than exposure near the ground level in the field.

TABLE 2.—*Weather conditions at Cass Lake, Minn., from July 1 to Aug. 27, 1936, as shown by readings taken at 5 p. m.*¹

Range of temperature	Occurrence	Range of relative humidity	Occurrence	Range of wind velocity	Occurrence
° C.	Days	Percent	Days	Miles per hour	Days
12-20	7	76-100	6	0-5	15
21-26	18	51-75	16	6-10	31
27-32	25	26-50	33	11-16	12
33-39	8	18-25	3	-----	-----

¹ Data from records in district ranger's office, Cass Lake, Minn.

The results of two tests conducted in 1934 afford evidence of the validity of machine tests. One of these was a test of the value of various foliage sprays in reducing drought mortality (16). A total of 49,000 seedlings of red pine, white pine, and white spruce (*Picea glauca*) were sprayed and planted in replicated blocks in the field. The percentage survival of these plants was compared with the relative drought resistance of sprayed seedlings of the same species as revealed by 12 tests made in the machine. The machine tests agreed with the field tests in indicating that one spray was slightly injurious and the others ineffective. During the same summer, preliminary experiments were begun to test the effects of fertilizers on drought resistance. The field and machine tests cannot be considered entirely comparable in this case, owing to great differences in soil volume and in moisture content; however, the results of machine tests were in general agreement with field survival.

Further evidence that the results of machine tests are truly indicative of capacity to resist natural drought was obtained through a series of field and machine tests carried out to determine the relative resistance of stock from two nurseries, the Lydick and the experimental.⁷ Most of the plant material used in this study was grown in these nurseries. During the summer of 1936 plantings were made in a dry exposed field on July 30 and August 11, at the height of the dry period, and the results of these plantings were compared with those of tests made between July 23 and September 17 in the 1933 machine. Two types of tests were made, wet and dry. For wet tests in the machine, the soil was saturated before the plants were placed in the machine but no water was added thereafter. The plants remained in the machine until dead. For wet tests in the field, made on August 11, 1 pint of water was poured around each seedling immediately after planting. For dry tests in the machine, seedlings were potted in air-dry soil, placed in the machine for from 4 to 6 hours, watered, and removed to a shaded bed in the nursery; for dry tests in the field, made on July 30, seedlings were planted in dry soil and were left overnight before being watered. All plants were examined daily to determine the number dead. The greatest differences in survival between the plants from the two nurseries, respectively, for dry tests in the machine and for both kinds of field tests, occurred between the second and the third week. The number surviving at this time was used as an index of relative resistance.

The results from tests of drought resistance by the two methods are shown side by side in table 3. Survival percentages for field tests are based on 25 plants, those for machine tests on from 10 to 20 plants. The results from all tests on red pine are in agreement, though that of one field test failed to show significance. Neither the machine-test results for white pine nor those for jack pine are in agreement. Either the differences between the plants from the two nurseries were slight or—what is more likely—they fluctuated during the season. Results of field tests on jack pine also were in disagreement. Both methods of testing gave results indicating that no consistent differences prevailed between the two lots of jack pine.

An attempt was made to repeat these tests during 1937. A test in early July gave a valid and consistent comparison, shown in table 3.

⁷ A description of these nurseries is given on p. 11.

A second test made in early August also gave a consistent comparison between machine and field survival; however, in this test the plants in the field received only a mild exposure to drought. (The 1937 reversal in relative drought resistance of stock from the two nurseries is explained in a later section.)

It may be stated that so far as evidence is available there is substantial agreement between results of machine tests of drought resistance and field survival during dry periods.

TABLE 3.—Comparison of results of machine tests with field survival of 2-year-old stock¹

Year and species	Soil condition at start of test	Survival in machine tests			Survival in field tests		
		Lydick stock	Experimental stock	Significance of difference ²	Lydick stock	Experimental stock	Significance of difference ²
1936 Red pine.....	Dry.....	Percent 20	Percent 60	0.04	Percent 52	Percent 68	0.30
	Wet.....	Days 4.7	Days 5.5	.02	28	68	.01
Northern white pine.....	do.....	5.2	6.1	.01			
	do.....	5.6	7.3	.01	8	56	.01
	do.....	5.3	4.6	.01			
	do.....	6.6	6.9	.30			
Jack pine.....	Dry.....	Percent 20	Percent 70	.01	36	64	.05
	do ³	27	60	.06			
1937 Red pine.....	Wet ³	Days 5.3	Days 4.7	.07	52	68	.30
	Moisture content 5 percent.	5.0	4.2	.01			
		6.7	4.3	.01	100	30	.01
					94	86	.01

¹ 2-0 stock except as noted; i. e., 2-year seedlings.

² Determined by Fisher's "F" test (4) for machine tests in wet soil, by chi-square test for all others. Only values of 0.05 or less are considered to indicate significance.

³ 1-1 stock; i. e., 1-year seedlings transplanted for 1 year before field planting.

IMPROVING DROUGHT RESISTANCE BY REGULATING MOISTURE SUPPLY

GREENHOUSE EXPERIMENTS

On April 24, 1936, 22 plants each of 3-year-old red pine, 3-year-old northern white pine, and 2-year-old jack pine were potted in 4-inch clay pots each containing approximately 1,000 grams of soil. The plants were watered daily during the next 10 days. Then the plants of each species were divided into two groups representing the same range of size and vigor. Thereafter one group was watered daily or twice daily to maintain the soil moisture between 18 and 22 percent. The second group was watered every third or, in extremely warm weather, every second day; this allowed the soil moisture to vary between 5 and 20 percent. The wilting coefficient of the greenhouse soil used was 3.4 percent. This treatment continued for 71 days. The pots were then brought to equal moisture content and placed in the 1936 drought machine, where each plant received daily two applications of 50 grams of water. The temperature in the machine was held constant at 44° C.; the relative humidity averaged 27.5 percent. For all three species, drought resistance was significantly higher in the plants that had been subjected to periodic soil dryness (table 4).

TABLE 4.—*Influence of exposure to periodic soil dryness on drought resistance of pine seedlings*

Date of test	Species and age class ¹	Total plants used	Length of treatment	Average survival in machine	
				Stock watered daily	Stock watered every third day
		<i>Number</i>	<i>Days</i>	<i>Days</i>	<i>Days</i>
July 14, 1936.....	Red pine (2-1).....	22	71	4.5	² 6.4
	Northern white pine (2-1).....	22	71	3.6	³ 4.6
	Jack pine (2-0).....	22	71	5.2	³ 7.3
June 21, 1937.....	do.....	120	30	5.0	5.2
July 20, 1937.....	do.....	80	60	4.5	³ 4.9
Jan. 10, 1938.....	Jack pine (1-0) ⁴	50	43	3.8	² 4.4
	do. ⁵	50	43	3.0	² 3.5

¹ In the age-class symbol, the first figure indicates years in seed bed; second figure, subsequent years in transplant bed.

² Values significantly greater at 0.01 level for periodic than for daily watering.

³ Values significantly greater at 0.05 level.

⁴ Soil not replaced.

⁵ Soil replaced.

The experiment was repeated in 1937 with 2-0 jack pine. Potted seedlings were watered uniformly for 60 days; they were then given differential watering like that in the preceding experiment, one lot for 30 days and one lot for 60 days. At the end of each period of treatment seedlings were transplanted from the clay pots into cans containing sand with a 5-percent moisture content, and placed in the 1936 machine. No water was added thereafter. The machine was operated at a temperature of 37° C. and a relative humidity of 20 percent. The seedlings that had been watered infrequently for 30 days showed a statistically insignificant advantage in resistance; for those that had been treated for 60 days, the difference was significant at the 0.05 level (table 4).

In a third experiment, 1-year-old jack pines were transplanted to 4-inch clay pots on September 15, 1937. These were watered daily until November 29; then two uniform lots of 50 plants each were selected for treatment. The lot that was to be watered infrequently was placed on a bare wooden table, to facilitate evaporation from the soil and pots; its soil moisture was allowed to fluctuate between 5 and 20 percent. The lot to be watered daily was placed on a greenhouse bench filled with moist sand; its soil moisture was maintained between 18 and 22 percent.

After 43 days of this treatment 25 plants of each lot were removed from the clay pots, shaken free of soil, and repotted in tin cans containing sand with a 5-percent moisture content. The pots containing the remaining plants were saturated with moisture and allowed to drain 1 hour; then each of the plants, with soil intact, was transferred to a tin can. No water was added during the test, which was made in the 1936 machine, operated at a temperature of 41.2° C. and a relative humidity of 10.8 percent. Treatment of the data by the analysis-of-variance method indicated that the plants infrequently watered had a highly significant advantage in drought resistance (table 4).

In addition to these experiments 19 other tests, involving a total of 988 plants, have been carried out to determine the effect of watering methods on drought resistance of coniferous seedlings grown in the greenhouse. The results, although not always statistically significant, have been in general agreement with those of the tests described.

NURSERY EXPERIMENTS

The practical value of controlled watering as an expedient for increasing drought resistance of coniferous seedlings was demonstrated in 1936 and in 1937 by large-scale experiments in two nurseries.

To understand clearly the significance of the several nursery tests, a few important differences between the two nurseries must be borne in mind. The Lydick, a 66-acre Forest Service nursery at Cass Lake, Minn., prior to 1937 had been producing stock less desirable in quality than that grown in the experimental nursery, located 5 miles to the southeast. The difference was ascribed to a variety of causes, of which the better soil in the experimental nursery and the method of watering used there were considered to be the more important. The soil in the Lydick nursery is a fine sand which, during 1936 and 1937, was greatly improved by heavy applications of compost. From the establishment of this nursery in 1933 until the spring of 1937 it was the nurserymen's practice to apply water liberally whenever the beds appeared dry. During the hot, dry period of 1936, in order to minimize losses the beds were watered daily. In 1937, artificial watering was reduced in an effort to produce more resistant stock.

The experimental nursery is located in a 3½-acre clearing surrounded by a 50-year-old aspen stand. Owing to the protection afforded by the timber and to the higher moisture-holding capacity of the fine sandy loam soil, this nursery does not require such frequent watering as the Lydick. As the purpose of establishing this nursery included simplification of care together with production of hardy planting stock, artificial watering has been held to a minimum. Normally, water is applied only during the germination period. In 1936, however, it was necessary to water during July and August, applying a total of 1.62 inches of water to first-year seedbeds and 0.32 inch to 2- and 3-year beds.

For reasons not fully understood, the jack pine stock from the experimental nursery hardens later in the fall than that from the Lydick. Consequently, if planted in the fall it suffers from winter injury.

TABLE 5.—*Field survival in 1936, by species and age class and by nursery of origin, of pine seedlings from the Lydick and experimental nurseries planted in the fall of 1935*

Date examined	2-0 jack pine		1-0 jack pine		2-0 red pine	
	Lydick	Experimental	Lydick	Experimental	Lydick	Experimental
	Percent	Percent	Percent	Percent	Percent	Percent
June 24.....	70	65	85	79	80	79
July 30.....	49	54	60	65	47	58
Aug. 11.....	37	47	50	54	30	46
Sept. 8.....	30	41	39	45	-----	40

The behavior of stock from the two nurseries when planted in the fall is illustrated by the field survival in a special test planting⁸ established in 1935, shown in table 5. The differences in survival of

⁸ Planned and executed by R. H. Blythe, Jr., and Paul Zehngraff.

red pine during the summer of 1936 are statistically significant. Jack pine stock from the Lydick nursery showed significantly greater losses during the summer than that from the experimental nursery. The Lydick stock had a higher survival over winter, however, and the difference in survival at the end of the 1936 season is not significant.

In addition to this experiment, about 60 acres were planted in the fall of 1935 with jack pine stock from the two nurseries. No attempt was made to lay out special comparative plots, since the planting covered irregular areas in the east tier of forties of a section. A count of survival made in August 1936 gave results shown in table 6. Stock from the experimental nursery showed a distinct advantage over that from the Lydick.

To check the reliability of the evidence that stock produced in the experimental nursery was better suited for field planting than that produced in the Lydick, the series of field and machine tests mentioned in an earlier section were carried out during 1936 and 1937. The quantity of water applied during the 30-day period preceding machine tests of red pine, and results with this stock, are shown in table 7. In each of the three tests made in 1936, stock from the experimental nursery proved to be more drought resistant than that from the Lydick. The quantity of water supplied to the Lydick stock preceding the first two tests was approximately three times the quantity supplied to the experimental stock. Artificial watering was discontinued in both nurseries during September, but increased drought resistance of experimental stock induced by watering methods used during July and August persisted.

TABLE 6.—*Field survival on Aug. 21, 1936, of 2-year-old jack pines from the Lydick and experimental nurseries planted the previous fall*

Forty No.	Lydick nursery stock		Experimental nursery stock	
	Age class	Survival	Age class	Survival
	<i>Years</i>	<i>Percent</i>	<i>Years</i>	<i>Percent</i>
32	2-0	28	1-1	55
31	2-0	36	1-1	59
22	2-0	35	2-0	58
21	2-0	19	2-0	51

The tests on seedling stock in 1937 showed Lydick stock to have greater resistance than experimental stock—a direct reversal of the 1936 results. However, the data for 1937 show relatively little difference between the two nurseries as to water supply. Presumably, because of the low moisture-holding capacity of its soil, less moisture was available in the second-year seedbeds of the Lydick nursery than in those of the experimental nursery, even though the former received artificial watering. Unfortunately no record of soil moisture is available. It is significant, however, that the Lydick nurseryman was making a deliberate effort to increase the hardness of his stock by limiting artificial watering. The fact that he was obliged to apply water whereas no artificial watering was needed in the experimental nursery is evidence of drier soil conditions in the Lydick. Furthermore, each time seedling stock was lifted for testing it was noticed that the Lydick beds were drier than the experimental beds.

TABLE 7.—*Drought resistance of red pine in 1936-37, by nursery of origin and by previous watering*

Class of stock and date of test	Total plants used	Watering ¹ during 30 days preceding test				Average survival in machine		Significance of difference
		Days water was applied		Total quantity of water applied		Lydick	Experimental	
		Lydick	Experimental	Lydick	Experimental			
1936								
2-year seedlings:	Number	Number	Number	Inches	Inches	Days	Days	
July 23.....	20	24	6	4.12	1.29	4.7	5.5	0.02
July 29.....	20	26	7	4.15	1.29	5.2	6.1	.01
Sept. 30.....	30	13	12	2.26	2.21	5.6	7.3	.01
1937								
July 15.....	40	16	9	6.92	5.33	5.0	4.2	.01
July 21.....	40	17	11	7.42	6.06	4.7	4.1	.01
July 27.....	40	17	10	8.32	6.85	6.3	4.6	.01
Aug. 16.....	40	11	7	2.53	1.81	6.7	4.3	.01
Sept. 9.....	40	11	6	6.22	5.50	8.2	5.2	.01
1937								
2-1 transplants:								
July 2.....	32	13	9	4.71	1.73	3.6	6.0	.01
July 2.....	32	13	9	4.71	1.73	3.6	5.2	.01
Aug. 1.....	32	19	9	12.58	6.87	5.3	6.4	.01
Sept. 2.....	32	10	8	5.74	5.36	4.1	5.8	.01

¹ Includes rainfall and artificial watering. Experimental nursery stock received only 0.32 inch of artificial watering in 1936 and no artificial watering in 1937.

The Lydick transplants tested in 1937 had been set out adjacent to first-year beds, where they received more frequent and abundant watering than the 2-year-old seedlings. These transplants proved far less drought resistant than similar stock from the experimental nursery which received no artificial watering.

The reversal in comparative drought resistance between seedlings and transplants is directly related to quantity of water received. Seedlings deliberately hardened in the Lydick nursery were more resistant than experimental seedlings, but transplants abundantly watered in the Lydick nursery were less resistant than experimental transplants. This is concrete evidence that drought resistance of coniferous seedlings can be improved by controlling the moisture supply in the nursery.

Three tests involving differential treatments applied within a single nursery may be cited to strengthen this conclusion. During 1936 one portion of a bed of 2-year-old red pine in the experimental nursery was allowed to go through the summer without artificial watering; the remainder of the bed received 0.32 inch of water, in seven applications. On the first of October, plants from both portions of the bed were tested in the 1933 drought machine. The plants from the watered portion of the bed survived an average of 6.2 days; those from the unwatered portion, 6.9 days. Since the probability that this difference was due to chance is less than 0.01, it may be considered highly significant.

In another experiment, 1-year-old jack pines were transplanted to plots in the Lydick nursery in the spring of 1935. All plots received

normal watering from the overhead sprinkling system. Half the plots received, in addition, a daily application of 0.4 inch from sprink-

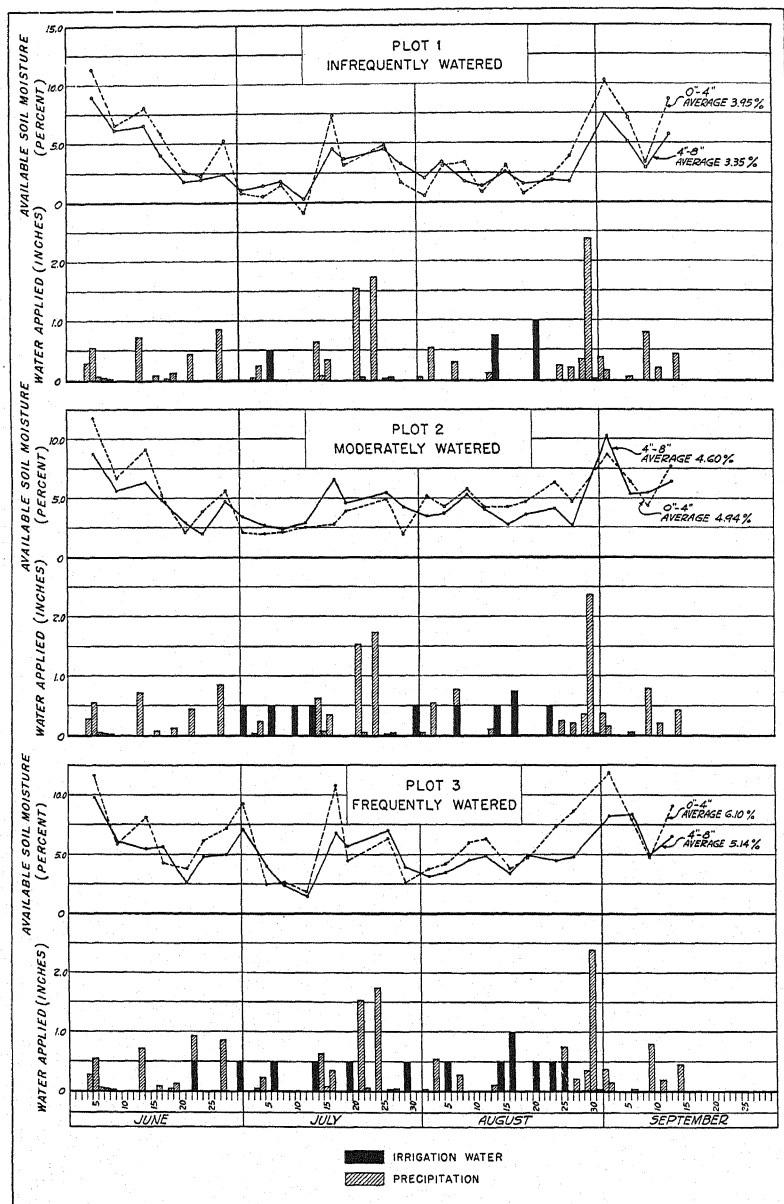


FIGURE 3.—Variations in soil moisture of three jack pine plots in the Hayward, Wis., nursery, during the growing season of 1937.

ling cans. After being thus treated for two growing seasons, the plants were tested in the 1933 drought machine. Plants from plots watered daily survived 4.8 days; plants from plots watered normally, 5.9

days. Though only 10 plants representing each treatment were tested, the difference was significant at the 0.02 level.

A third experiment,⁹ also involving jack pine, was conducted at the Forest Service nursery at Hayward, Wis., during the summer of 1937. In this case the moisture content of the soil was determined at biweekly intervals. The soil in this nursery was a medium sand having a wilting coefficient of 3.5 percent. Water was applied to three plots with a sprinkling can. The quantity of water applied, and the moisture available in the soil throughout the growing season, are shown graphically in figure 3. The soil moisture of the three plots showed similar seasonal trends. At critical periods the soil of the infrequently watered plot was always the driest. Of the 27 soil samples from this plot, 13 had less than 2.5 percent available moisture. A test of 26 seedlings from each of the three plots was made in the 1936 drought machine on October 25, the results of which are given in table 8. The infrequently watered plants were again significantly more resistant than those receiving a more abundant moisture supply.

TABLE 8.—*Effect of watering treatments in the Hayward, Wis., nursery in 1937 on drought resistance of 2-0 jack pine*

Watering treatment	Average available soil moisture at 0- to 8-inch level	Survival in machine
	Percent	Days
Infrequent.....	3.6	17.9
Moderate.....	4.8	6.6
Frequent.....	5.6	6.7

¹ Significantly greater at 0.01 level.

PERMANENCE OF INDUCED DROUGHT RESISTANCE

Obviously, building up drought resistance in nursery stock by controlled watering would result in no practical gain unless the improved resistance persisted for at least one season after treatment.

Tests in 1936 having shown that controlled watering in the experimental nursery had induced greater drought resistance in 2-year-old red pine than that occurring in similar Lydick stock, experiments were carried out in 1937 on plants from the same beds to discover whether this difference was still retained, independent of the growing conditions prevailing during that year. On May 14, 2- and 1-year-old seedlings grown in both nurseries were transplanted in randomized rows in four replicate beds in each nursery. The beds in the Lydick nursery were located adjacent to first-year seedbeds, where they received during the growing season a total of 7.7 inches of water from an overhead sprinkling system; the beds in the experimental nursery received no artificial watering. Samples from each bed were lifted at monthly intervals and brought to St. Paul for testing in the 1936 drought machine. At St. Paul each consignment was divided into halves, which were tested separately, the second being stored at 5° C. until the test of the first was completed. A temperature of 36° C. was maintained throughout these tests, and a relative humidity of from 19 to 25 percent. The

⁹ Planned and executed by J. H. Stoeckeler and J. W. Jay, Lake States Forest Experiment Station.

results from each test were analyzed by the variance method to determine the significance of differences. Averages of survival in the machine are shown in table 9.

TABLE 9.—*Drought resistance of red pine nursery stock transplanted¹ in May 1937, in days of survival in drought machine*

Date lifted, 1937	Date machine test began, 1937	Plants per treatment	Stock lifted in third year from—				Stock lifted in second year from—			
			Lydick nursery, first two years in—		Experimental nursery, first two years in—		Lydick nursery, first year in—		Experimental nursery, first year in—	
			Lydick	Experimental	Lydick	Experimental	Lydick	Experimental	Lydick	Experimental
		Number	Days	Days	Days	Days	Days	Days	Days	Days
May 1	May 4	30	6.18	-----	-----	2.97	3.66	-----	-----	3.20
May 1	May 12	30	5.32	-----	-----	2.99	3.94	-----	-----	2.79
June 1	June 3	25	3.48	-----	-----	4.51	4.47	-----	-----	3.80
July 1	July 6	28	3.61	4.45	4.06	6.04	2.87	2.74	2.93	3.06
July 1	July 13	28	3.65	4.61	3.76	5.16	3.06	2.70	2.52	2.69
July 30	Aug. 1	28	5.27	5.58	5.70	6.38	4.95	4.98	4.82	5.14
July 30	Aug. 11	28	4.46	4.88	4.76	5.72	3.92	4.59	4.36	4.57
Sept. 1	Sept. 2	28	4.13	4.69	5.57	5.82	4.08	4.31	4.17	4.58
Oct. 1	Oct. 5	28	5.69	7.02	5.79	6.12	5.55	5.00	5.16	4.37
Oct. 1	Oct. 14	28	6.09	6.70	5.88	6.45	5.47	5.32	5.84	5.27

¹ With the exception of plants used in May and June tests, which were lifted directly from seedbeds.

Of the seedlings lifted on May 1, those from the Lydick nursery were more resistant. As this was at the beginning of vegetative activity, results with this lot are of interest only as indicating seasonal variation. By June 1, stock from the experimental nursery was more resistant.

The first tests of transplanted stock were made on July 6. The 3-year-old stock from the experimental nursery, which by actual test was known to have been more drought-resistant than Lydick stock in 1936 (table 7), was significantly more resistant in 1937 than Lydick stock treated in the same way during the third year. The second-year stock not tested in 1936 showed insignificant differences in 1937.

This experiment clearly indicates that increased drought resistance brought about in 2-year-old red pine seedlings by control of water supply in the nursery persists during the third year. Whether treatments applied to seedlings during their first year can affect significantly their drought resistance during the second year remains an open question. Inasmuch as the ratio of the quantity of tissue developed during the first year to that developed during the second year is much less than the ratio of second-year tissue to third-year tissue, it is not surprising that no clear-cut evidence of a carry-over of resistance by 1-year-old seedlings was detected.

The advantage of the experimental nursery watering regime over that maintained in the Lydick nursery is further demonstrated by this experiment. Stock that had been transplanted at the beginning of the third year from the Lydick nursery to the experimental nursery (table 9, column 6 stock) showed significantly greater drought resistance, in six out of seven tests, than similar stock transplanted within the Lydick nursery (column 4). Conversely, experimental stock transplanted to the Lydick nursery (column 5) showed significantly

less drought resistance, in five out of seven tests, than similar stock transplanted within the experimental nursery (column 7). Also, Lydick stock transplanted at the beginning of the second year to the experimental nursery (column 10) became more resistant than similar stock transplanted within the Lydick nursery (column 8).

SUPPLEMENTAL FINDINGS

EFFECT OF OVEREXPOSURE TO SOIL DRYNESS

Although the drought resistance of conifers can be increased by exposure to soil dryness, caution is necessary to avoid injuring the plants by this treatment. An example of the effect of overexposure is afforded by two experiments made in the spring of 1937. Jack pine seedlings 1 year old were transplanted to dry sand in the late afternoon and allowed to stand overnight. The following morning the soil was saturated with moisture. In the first experiment the seedlings were given one, two, three, four, and five exposures to dry sand on consecutive days. Half of each lot of seedlings was tested the next day after all these treatments were completed. Drought resistance decreased as number of exposures increased (table 10). The remaining half was tested after being given 24 days of normal watering in which to recover. All except those that had been given five exposures survived 6 days in the machine, which presumably represents the equivalent of their resistance before treatment. In the second experiment, the plants were left overnight in air-dry sand, then watered and allowed 16, 8, 4, and 1 day to recover before being tested. Control plants were transplanted to moist soil. Differences in survival resulting from differences in time allowed for recovery were not significant, nor were differences in survival among the control lots; but the plants transplanted to dry sand showed significantly less resistance than the control plants.

TABLE 10.—*Decrease in drought resistance of 1-year-old jack pine seedlings resulting from exposure of roots to dry sand ¹ at night*

Nights of exposure	Days of recovery	Plants treated	Survival in machine	
			Initial test ²	Test 24 days later ³
<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Days</i>	<i>Days</i>
1	4	32	4.6	6.1
2	3	32	3.9	6.4
3	2	32	3.7	6.3
4	1	32	3.3	6.1
5	0	32	3.1	4.8
0	-----	32	5.9	6.2

¹ The plants not exposed to dry sand (for which data are given in last line) were drawn from the same lot of dormant seedlings but were tested 38 days later than the treated plants.

² Differences greater than 0.74 are significant at the 0.05 level, according to Fisher's (4) analysis-of-variance method.

³ Differences greater than 1.1 are significant at the 0.05 level.

In 1934 1-year-old jack pine and 2-year-old red pine seedlings were transplanted to a dry, sandy field, where they remained 2 months. By the end of that time more than half of them had died, and the remainder had been stunted in growth. The drought resistance of the more vigorous survivors was compared with that of fresh stock

from the nursery. The seedlings from the field proved to be markedly less resistant than the nursery stock.

It is concluded that after severe exposure the plants do not regain their original resistance unless allowed a rather prolonged period of recovery.

ROOT DEVELOPMENT AND DROUGHT RESISTANCE

The objectives of the experiments did not include determination of the influence of root development on drought resistance; however, some observations made on this point are worthy of consideration. Plants subjected to moderate drought tended to develop relatively more extensive root systems and smaller tops than those grown in soil continuously moist. In general, plants with well-developed roots were more resistant to drought than those with less extensive root systems. Yet this was not always the case; among the red pines tested in 1937, the more resistant were not always those with either the better-developed roots or the greater root-top ratios (table 11). These facts are presented not for the purpose of belittling the advantages of a good root system during periods of drought but rather to indicate that drought resistance in young conifers does not depend solely upon size of root system or relative root development.

TABLE 11.—*Drought resistance and root development of red pine transplants from both nurseries*

Nursery of origin and date of test, in 1937	Dry weight of roots		Root-top ratio		Survival in machine	
	Lydick	Experimental	Lydick	Experimental	Lydick	Experimental
Lydick:	<i>Grams</i>	<i>Grams</i>			<i>Days</i>	<i>Days</i>
Aug. 1.....	0.25	0.20	0.28	0.30	4.85	5.23
Sept. 1.....	.57	.50	.21	.17	4.13	5.57
Oct. 1.....	.60	.90	.32	.46	5.89	5.83
Experimental:						
Aug. 1.....	.76	.71	.36	.28	5.23	6.05
Sept. 1.....	1.12	.77	.30	.22	4.69	5.82
Oct. 1.....	.89	2.10	.34	.61	6.86	6.28

RELATIVE RESISTANCE OF DIFFERENT SPECIES

The experiments reported were not designed to test difference among species. This probably varies a great deal, according to the treatment received by the plants previous to the survival test. However, from the data in table 4 it is seen that jack pine is the most resistant, followed by red pine and white pine. This applies only to stock of about the same size and in the same stage of vegetative development. Further discussion of this question is deferred until more easily interpreted data are available.

DORMANCY AND DROUGHT RESISTANCE

Resistance to drought varies widely depending upon vegetative activity. This is clearly evident from the survival of plants from the same beds tested at several different times during the course of a season (table 9). Before the beginning of active growth in the spring, drought resistance was relatively high. With the beginning of stem

elongation, drought resistance showed a pronounced decrease, which persisted until active growth ceased in early summer. Thereafter, drought resistance increased.

These tests, together with others not reported in this paper, indicate that the drought resistance of actively growing conifers differs widely from that attained after a short period of hardening. Because of this difference, results of tests made on actively growing stock are likely to be wholly inconsistent with results of tests on similar stock after vegetative activity has declined. It has been found that drought resistance can be most effectively tested after rapid height growth has ceased, during the period in which the newly formed tissue is maturing but before the plant becomes dormant. This is the season at which droughts are most likely to occur.

DISCUSSION

The fact that the relative survival of coniferous seedlings in the drought chamber was in substantial agreement with that in the field during periods of drought clearly justifies the use of artificially produced drought in studies of the drought resistance of conifers. The advantages of machine tests over field survival tests are many: (1) Tests in the machine are free from biotic influences which often disturb tests made in the field. (2) The machine is available for tests at any time, whereas field tests can be made only during dry periods. (3) The close control over environmental factors possible in the machine greatly reduces variability, with consequent improvement in the reliability of results. (4) Tests in the machine are of relatively short duration, hence are admirably suited for study of the fundamental nature of drought resistance. (5) Direct tests in the machine can be duplicated by workers in other regions, and this makes possible a concerted attack upon the intricate problem of drought resistance in plants.

It is concluded from the experiments described that drought resistance of Lake States pine seedlings can be improved by controlling the moisture supply in the nursery, that such control is a simple and practical expedient available in all large-scale nurseries, and that drought resistance induced in 2-year-old conifers by controlled watering persists to an appreciable extent during the third year. It should be emphasized, however, that drought resistance of young conifers depends upon a number of factors, of which moisture supply is only one. Drought resistance is known to be in part associated with region of seed origin (11), and preliminary experiments have indicated that it is influenced to an important degree by both light and soil fertility. Doubtless other factors that modify plant structure or nutrition will ultimately be found to affect the intricate internal balance which determines a plant's resistance to drought. Before comprehensive recommendations can be made to the practicing nurseryman, the action and interaction of the factors mentioned must be more thoroughly understood. More must be known, also, of the rate at which changes in drought resistance take place within the plant. Of this important subject little is known at present except that such changes take place rapidly in actively growing plants and very slowly in dormant plants.

SUMMARY

In order to test the drought resistance of potted coniferous seedlings, an improved "drought machine" in which artificial drought can be produced at will was devised. This consists of a thermostatically controlled, illuminated plant chamber with apparatus for drying air over calcium chloride and forcing it through the chamber. The relative drought resistance of different lots of coniferous seedlings as revealed by tests in this machine was found to correspond substantially with the relative field survival of similar lots during periods of drought.

The drought resistance of seedlings of Lake States pines, namely, red pine (*Pinus resinosa*), northern white pine (*P. strobus*), and jack pine (*P. banksiana*), was increased by subjecting the seedlings to moderate soil drought during the period of vegetative activity. This treatment tended to result in greater development of roots in proportion to top, and in smaller size of tops; but improved resistance did not depend solely upon size of root system or upon ratio between roots and top. Resistance built up in red pine through controlled watering during the second year in the nursery persisted to a significant degree during the following season.

Exposure to severe dryness of soil temporarily weakened the plants and, unless followed by an ample period of recovery, rendered them more susceptible to drought.

Improvement of drought resistance of Lake States pine seedlings by controlling the moisture supply in the nursery was demonstrated to be a practical procedure which can be applied in large forest nurseries.

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INHERITANCE STUDIES IN THE INTERSPECIFIC CROSS *Solanum demissum* LINDL. \times *S. tuberosum* L.¹

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INTRODUCTION

Hybrids between *Solanum demissum* Lindl. and *S. tuberosum* L. have been used for the purpose of securing commercially valuable varieties immune to late blight and having some tolerance to frost. The two species differ in chromosome number. *S. demissum* has been reported to have 36 haploid and 72 diploid chromosomes, with normal meiotic divisions (12, 21, 22, 30).³ Varieties of *S. tuberosum* have been reported to have 24 haploid and 48 diploid chromosomes, with various abnormalities in the meiotic divisions (1; 5; 6; 7; 8; 11; 12; 13; 14; 23, p. 113; 24; 30; 31; 33). This difference in chromosome number and the reversion of the hybrids to the *S. demissum* parent reported by Reddick (17, 18) suggested the need for a study of the cytology and the breeding behavior of the hybrids.

THE PARENT FAMILIES

The parents of the interspecific cross on which this work is based were a true breeding form of *Solanum demissum* form *xitlense* Buk., seed of which was originally obtained from Dr. S. M. Bukasov from the Union of Soviet Socialist Republics in 1931, and *S. tuberosum* selection Minnesota 4-39-2-2, a free-flowering, pollen-fertile, fourth-generation inbred seedling of U. S. D. A. 14329, which was a cross of Keeper \times Silverskin. The cross was made in 1932 by using *S. demissum* as the pistillate parent.

Thirty-one seedlings of each parent were grown in 1936 for comparison with each other and with their hybrid progeny. The term "parents," as used throughout this paper, refers to these selfed seedling progenies, not to the clones. The parents differed from each other in many characters, a few of which were measured, the means with their standard errors being compared in table 1.

The cotyledons of *Solanum demissum* were much narrower than those of *S. tuberosum*, but no shorter, and the width-over-length or shape index of *S. demissum* was, therefore, much smaller. Terminal leaflets of *S. demissum* also were narrower in proportion to length than those of *S. tuberosum*, though not as markedly so as the cotyledons.

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² The writer is indebted to Dr. F. A. Krantz, of the Minnesota Agricultural Experiment Station, for supplying the material and for suggestions in connection with this investigation.

³ Italic numbers in parentheses refer to Literature Cited, p. 37.

They were much smaller in *S. demissum*. Mature leaves of *S. demissum* were only about one-fourth as large in area as the mature leaves of *S. tuberosum*. Stems of *S. demissum* were about one-third as long as those of *S. tuberosum*; internodes were less than one-fourth as long; and stem diameters were one-half as large. Stems of *S. demissum* were all prostrate, while of the plants of *S. tuberosum* seven were classed as erect, five as semierect, and nine as semiprostrate. *S. demissum* is reported to have stolons as much as a yard long (20), but at University Farm the few tubers that were set were on stolons approximately 30 cm. long, while stolons of *S. tuberosum* were 5 cm. long. *S. demissum* does not set tubers at University Farm until late in the fall and had set practically no crop by November 1, while *S. tuberosum* had set a fair crop of medium-sized tubers by October 1.

TABLE 1.—A comparison of progenies from selfed seed of *Solanum demissum* and *S. tuberosum* selection 4-39-2-2

Character	<i>S. demissum</i>		<i>S. tuberosum</i> 4-39-2-2	
	Number of plants	Mean value for character ¹	Number of plants	Mean value for character ¹
Morphological characters:				
Cotyledon width..... millimeters..	31	3.48±0.11	31	6.00±0.25
Cotyledon length..... do.....	31	8.06±.33	31	7.90±.33
Cotyledon shape index.....	30	.44±.02	29	.75±.03
Terminal-leaflet width..... millimeters..	27	10.96±.60	20	25.40±1.46
Terminal-leaflet length..... do.....	27	17.70±1.10	20	32.75±1.80
Terminal-leaflet shape index.....	27	.68±.01	20	.78±.02
Mature-leaf area..... square centimeters..	26	6.40±.52	20	28.50±3.96
Mature-stem height..... centimeters..	27	16.41±.94	21	47.50±2.54
Stem diameter..... millimeters..	26	3.93±.12	20	7.53±.34
Internode length..... do.....	27	6.31±.38	21	28.09±1.22
Tubers..... number.....	18	7.20±2.65	24	24.54±4.58
Weight of largest tuber..... grams..	26	1.30±	24	10.70±2.58
Stolon length..... centimeters..		30.00±	23	4.97±.94
Physiological characters:				
Heat and drought injury ²	31	1.87±.12	28	1.04±.22
Immaturity ³	26	5.00±.00	22	.27±.19
Frost injury ³	26	.00±.00	2	5.00±.00

¹ Standard error is used rather than probable error.

² Arbitrary scale, 0-3.

³ Arbitrary scale, 0-5.

Heat and drought affected *Solanum demissum* more than *S. tuberosum*, all plants of *S. demissum* being injured by the severe heat and drought of July 1936, while 12 of the 28 plants of *S. tuberosum* escaped injury. Plants of *S. tuberosum* matured in early September, while all plants of *S. demissum* were still green during October. Plants of *S. demissum* were uninjured by the first frost of the year on October 1, which killed the two plants of *S. tuberosum* selection 4-39-2-2 immature enough to show frost injury, and all plants of another *S. tuberosum* family growing in the same plot, as well as pepper and eggplants in adjoining plots. *S. demissum* is reported to withstand temperatures of about 27° F. (2, 3, 4, 9, 16, 17, 27, 32) and to be late-blight-immune (3, 4, 10, 14, 17, 18, 19, 20, 25, 26, 27, 28, 29).

THE F₁ FAMILY

The 15 plants making up the F₁ family were started in the greenhouse in the early spring of 1933 and grown there for 4 months before being moved to the field. They were alike in general appearance and

were intermediate in type between the two parents. Hybrid vigor was especially noticeable in the large leaves and the large showy purple flowers.

Several F_1 hybrids of *Solanum demissum* by *S. tuberosum*, similar in their general habit of growth and characters to the F_1 described above, were grown in 1936. The *S. tuberosum* parents of these F_1 plants were not related to *S. tuberosum* selection 4-39-2-2, the parent of the F_2 and backcross plants used in this study. Characters of these F_1 plants are shown in tables 8 to 20 for comparison with characters of plants of the F_2 families.

Chromosome counts made from root tips of some of the F_1 plants of *Solanum demissum* \times *S. tuberosum* selection 4-39-2-2 showed 60 chromosomes, as would be expected from the union of 36 in the ovule of *S. demissum* with 24 in the pollen grain of 4-39-2-2.

A study was made of the chromosome behavior in the pollen mother cells of anthers imbedded in paraffin, sectioned 10 μ thick, and stained with crystal violet.

Meiosis in the hybrids was irregular. At diakinesis various chromosome groupings, including rings of four or more chromosomes, long chains of terminally united chromosomes, and what appeared to be trivalent chromosomes were seen.

At metaphase I, 6 of the 61 cells examined in side view appeared to be normal, with all the chromosomes lined up on the metaphase plate. No univalents or trivalents could be detected. Fifty-five cells, or 90 percent, had one or more groups of nonoriented chromosomes, as shown in table 2. The greatest number of nonoriented chromosomes found in one cell was 6. These often showed signs of splitting, and they were frequently as large as the largest bivalents on the plate. Presumably, however, they were for the most part univalents rather than bivalents.

TABLE 2.—Pollen mother cells in the F_1 hybrid showing nonoriented chromosomes at metaphase I and II, lagging chromosomes at anaphase I and II, and micronuclei at telophase II, with the number of chromosomes or chromosome groups involved in each cell

Item	Cells showing described behavior in indicated number of chromosomes									
	Number chromosome involved									Total cells
	0	1	2	3	4	5	6	7	8	
Metaphase I: Cells showing nonoriented chromosomes....	No. 6	No. 22	No. 20	No. 9	No. 1	No. 2	No. 1	No. 1	No. 1	No. 61
Anaphase I: Cells showing lagging chromosomes.....	---	9	36	7	20	2	4	1	2	81
Metaphase II: Cells showing nonoriented chromosomes....	21	23	19	11	4	5	4	1	---	88
Anaphase II: Cells showing lagging chromosomes.....	190	18	15	3	---	---	---	---	---	226
Telophase II: Cells showing micronuclei.....	76	27	15	3	1	---	---	---	1	123

Trivalent associations were extremely rare either on the metaphase plate or among the nonoriented chromosomes, though a few were seen, as well as one chain of four. Nonoriented groups of two or three chromosomes were occasionally seen.

At anaphase I, all of the 81 cells examined showed lagging chromosomes. In some cases the lagging chromosome was clearly a univalent,

and rarely a lagging trivalent was found, but most of the lagging chromosomes had the appearance of conjugated pairs. Presumably these last were univalents which were splitting. The number of lagging chromosomes or chromosome groups found in each of the 81 cells examined in side view is given in table 2.

At telophase I some of the lagging chromosomes had a faded appearance and seemed to be disintegrating in the cytoplasm. At interkinesis the chromosomes were well separated, with no suggestion of secondary association.

At metaphase II a somewhat smaller proportion of the cells showed nonoriented chromosomes than at metaphase I, as is shown in table 2. Some of the chromosomes which lay outside the spindle at the first division apparently either were recovered at interkinesis or had disintegrated before the second division.

Few lagging chromosomes were observed at anaphase II, but there were numerous nonoriented chromosomes scattered throughout the cells, often near the cell walls. Forty-six cells in a total of 226 observed showed nonoriented chromosomes. The number of lagging chromosomes found in these same 226 cells, or 452 division figures, is shown in table 2.

Micronuclei were counted at telophase II, and the number of micronuclei found in each of the 123 cells observed is shown in table 2. There were surprisingly few, considering the number of non-oriented and lagging chromosomes observed at earlier stages. Probably many of the chromosomes which were cast out during the first division or in early stages of the second division were recovered at anaphase of the second division, and it is possible that at least some of the nonoriented chromosomes were lost through degeneration. The latter possibility is supported by the fact that there were no micronuclei to be found in the quartets or young pollen grains. It is unlikely that micronuclei present at telophase II would become reincorporated into the pollen nuclei.

Pollen resulting from these irregular divisions varied considerably in size, the smallest grains making up 5 to 10 percent of the total. These smallest grains and some of the medium-sized grains were empty or contained partially disintegrated protoplasm. The largest grains, making up approximately one-third of the total, had the dense and evenly granulated protoplasm characteristic of good pollen. Stainability with acetocarmine of the pollen of some of the F_1 plants is shown in table 3. It was fairly high, considering the numerous irregularities found in meiosis, ranging from 14.8 to 30.8 percent in the field at University Farm, from 23.9 to 45.2 percent in the greenhouse, and above 50 percent in the field at Castle Danger on the north shore of Lake Superior, where environmental conditions usually favor flowering and fruit setting in the potato. In the greenhouse there was as much variation between different pots of an F_1 clone as between different clones.

The relationship of pollen stainability to germination on agar is shown in table 4. Flowers from each of three different F_1 plants and from *Solanum demissum* were used. Pollen of each flower was first shaken onto an agar gel containing 1 gram of agar and 20 grams of sugar to 100 cc of water. The remaining pollen was examined in acetocarmine. In the *S. demissum* flower 61.3 percent of the pollen was stainable and

26.8 percent germinated, indicating that nearly half of the stainable pollen was viable on agar. Pollen of the F_1 plants behaved very differently, though some pollen of each flower germinated. Stainability averaged 59.3 percent, and germination only 2.3 percent. Grains that germinated were all of the largest size.

TABLE 3.—Stainable pollen in different F_1 plants of *Solanum demissum* \times *S. tuberosum* selection 4-39-2-2

Location, date, and plant breeding No.	Flowers	Mean percent of stainable pollen ¹	Location, date, and plant breeding No.	Flowers	Mean percent of stainable pollen ¹
University Farm, summer of 1933:	Number	Percent	Greenhouse, Mar. 5 to 17, 1934—Continued.	Number	Percent
1.....	3	20.0 \pm 6.1	7, pot b.....	10	31.1 \pm 2.0
4.....	5	30.8 \pm 2.8	7, pot c.....	13	32.1 \pm 1.5
8.....	3	29.0 \pm 8.7	7, pot d.....	11	34.5 \pm 2.7
9.....	5	14.8 \pm 2.1	10, pot a.....	7	28.7 \pm 4.6
10.....	2	29.5 \pm 7.5	10, pot b.....	7	37.0 \pm 3.5
11.....	2	26.5 \pm .5	10, pot c.....	4	37.7 \pm 1.4
Greenhouse, Mar. 5 to 17, 1934:			10, pot d.....	6	45.2 \pm 1.4
3.....	15	23.9 \pm 2.4	Castle Danger, July 27, 1934:		
4.....	1	31.0 \pm ---	12.....	5	56.0 \pm 5.0
5, pot a.....	11	28.5 \pm 3.0	13.....	5	55.0 \pm 4.4
5, pot b.....	7	33.4 \pm 3.8	14.....	5	50.4 \pm 3.7
7, pot a.....	9	24.0 \pm 2.2			

¹ 100 pollen grains of each flower were counted. Standard error is used rather than probable error.

TABLE 4.—Relation between stainability of the pollen of *Solanum demissum* and its hybrids and its germination on agar

Item	Stainable grains		Total	Germinated grains		Total
	Number	Percent	Number	Number	Percent	Number
<i>S. demissum</i>	676	61.3	1,102	327	26.8	1,219
F_1 , flower 1.....	170	68.0	250	8	1.6	500
F_1 , flower 2.....	158	63.2	250	8	1.6	500
F_1 , flower 3.....	117	46.8	250	18	3.6	500
F_1 mean.....		59.3			2.3	

Most of the F_1 plants did not set selfed seed, although numerous hand pollinations were made both in the greenhouse and in the field at University Farm. The only plant that set selfed seed in the greenhouse was plant 15, which was also the only one that died without forming tubers. Plants 1 to 11 were grown at University Farm in 1934 and did not set selfed seed. Plants 12, 13, and 14 were moved from the greenhouse to the potato-breeding plots at Castle Danger, where they grew vigorously and blossomed freely, having a succession of flowers throughout the season. The pollen was over 50 percent stainable. Plant 12 was estimated to have produced seed from not more than 1 percent of its flowers, and plant 13 was even less fruitful. Plant 14, which bloomed most profusely, set almost no seed. The naturally set seed of plants 12 and 13 was used to grow the F_2 families 12 and 13.

THE F_2 FAMILIES

In 1934 F_2 families 12 and 15 were grown from seed of F_1 plants 12 and 15. The seed from which F_2 family 15 was grown was produced in the greenhouse by self-pollination. The 103 plants of F_2

family 15 were uniform and practically indistinguishable from the *Solanum demissum* parent. No tubers were obtained from this family, but 90 of the plants set seed. The 90 F_2 families grown in 1936 were also practically indistinguishable from *S. demissum*.

F_2 family 12 was grown from seed obtained from the naturally set fruit of F_1 plant 12. Chromosome counts were obtained from the root tips of 15 of the plants of this family. None of the plants had more than 58 chromosomes, 2 less than the number in the F_1 . There was no apparent relationship between chromosome number and either tuber formation or seed production. The approximate chromosome counts of those that set seed as well as tubers were 49, 50, 51, and 56; of those forming only tubers but no seed 50, 53, 54, 56, and 58; and of those forming neither tubers nor seed 48, 52, 53, 54, 56, and 58.

In 1936 F_2 family 13 and a further lot of F_2 family 12 were grown from seed of naturally set fruit produced at Castle Danger by F_1 plants 12 and 13. These two families were similar to each other and resembled *S. tuberosum* in general appearance. Several morphological and physiological characters were measured. The results are shown in tables 5 to 20.

MORPHOLOGICAL CHARACTERS

The range in cotyledon width is shown in table 5. Cotyledons of the F_2 plants varied from narrower than those of *Solanum demissum* to as wide as the widest of *S. tuberosum*. The range in cotyledon length is shown in table 6. Cotyledons of the F_2 plants varied from shorter to longer than the cotyledons of the two parent families. The larger standard deviations show that this greater range was due to greater variability in cotyledon length among the F_2 plants and not to the fact that more F_2 than parent plants were measured. The range in shape indices, width divided by length, is shown in table 7. Indices of the F_2 plants ranged from as low as the lowest index of *S. demissum* plants to as high as the highest index of plants of *S. tuberosum*, with the mean indices half way between the means of the parent families. This distribution is to be expected if the character is determined by a number of factors.

TABLE 5.—Distribution of individuals in the parent and F_2 families according to cotyledon width

Family	Plants having cotyledons of a width—								Total plants	Mean width of cotyledons ¹
	1 mm.	2 mm.	3 mm.	4 mm.	5 mm.	6 mm.	7 mm.	8 mm.		
	Number	Number	Number	Number	Number	Number	Number	Number	Number	Milli-meters
<i>Solanum demissum</i>	2	20	8	1	2	14	9	2	31	3.5±0.1
<i>S. tuberosum</i> 4-39-2-2.....	1	2	1	2	1	10	1	1	31	6.0±.3
F_2 12.....	2	2	7	19	14	10	1	1	56	4.4±.2
F_2 13.....	5	3	7	14	15	5	1	-----	50	4.0±.2

¹ Standard error is used rather than probable error.

Terminal leaflets of both F_2 families were larger than those of either of the parents, as is shown in table 8, but smaller than those of F_1 plants. Shape indices of the terminal leaflets, shown in table 9,

ranged from the lowest of *Solanum demissum* to almost the highest of *S. tuberosum* with the means half way between the means of the parents, as was true of the cotyledon shape indices.

TABLE 6.—Distribution of individuals in the parent and F_2 families according to cotyledon length

Family	Plants having cotelydons of a length of—											Total plants	Mean length of cotyledons ¹	Standard deviation
	2mm.	3mm.	4mm.	5mm.	6mm.	7mm.	8mm.	9mm.	10mm.	11mm.	12mm.			
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	Num-	Milli-	
<i>Solanum demissum</i>	-----	-----	-----	2	6	3	7	6	4	3	-----	31	8.1±0.3	1.8
<i>S. tuberosum</i> 4-39-2-2.....	-----	-----	2	2	3	4	8	7	2	3	-----	31	7.9±.3	1.8
F ₂ 12.....	2	1	1	4	8	11	11	6	6	4	2	56	7.5±.3	2.3
F ₂ 13.....	3	3	3	7	8	3	8	2	7	2	4	50	7.1±.4	2.7

¹ Standard error is used rather than probable error.

TABLE 7.—Distribution of individuals in the parent and F_2 families according to cotyledon shape

Family	Plants having indicated shape index (cotyledon width divided by length)														Total plants	Mean shape index of cotyledons ¹
	0.40	0.45	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00			
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	Number		
<i>Solanum demissum</i> -----	9	13	7	---	1	---	---	---	---	---	---	---	---	30	0.44±0.01	
<i>S. tuberosum</i> 4-39-2-2-----	---	---	1	---	1	1	4	10	3	1	5	---	3	29	.76±.03	
<i>F</i> ₂ 12-----	1	3	12	4	14	6	7	3	1	2	2	---	---	55	.60±.02	
<i>F</i> ₂ 13-----	3	1	15	1	9	5	5	3	3	2	1	---	2	50	.61±.02	

¹ Standard error is used rather than probable error.

TABLE 8.—Terminal leaflet length and width of the parent families, a group of F_1 plants, and the F_2 families

Family	Leaflets measured	Mean width ¹	Mean length ¹	Family	Leaflets measured	Mean width ¹	Mean length ¹
	Number	Milli-meters	Milli-meters		Number	Milli-meters	Milli-meters
<i>Solanum demissum</i>	27	11±1	18±1	F_2 12.....	50	32±1	45±2
<i>S. tuberosum</i> 4-39-2-2.....	20	25±2	33±2	F_2 13.....	42	28±2	40±2
F_1	15	35±7	53±10				

¹ Standard error is used rather than probable error.

TABLE 9.—Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to terminal-leaflet shape

Family	Plants having indicated shape index (Terminal leaflet width over length)												Total plants	Mean shape index of terminal leaflets ¹
	0.50	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00			
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.		
<i>Solanum demissum</i>	1	2	8	8	4	3	1	---	---	---	---	27	0.63±0.01	
<i>S. tuberosum</i> 4-39-2-2.....	---	---	1	1	2	3	5	5	2	---	1	20	.78±.02	
F ₁	---	---	3	6	4	2	---	---	---	---	---	15	.65±.01	
F ₂ 12.....	1	---	6	6	9	15	9	2	1	1	---	50	.70±.01	
F ₂ 13.....	2	2	6	5	6	13	3	4	1	---	---	42	.69±.01	

¹ Standard error is used rather than probable error.

Areas of mature compound leaves were measured in square centimeters. Leaves of both F_2 families were larger on an average than those of the *Solanum tuberosum* parent, though considerably smaller than those of the F_1 plants, and the range was from the smallest leaves of *S. demissum* to almost twice the size of the largest leaves of the *S. tuberosum* parent, as shown in table 10. Leaves of *S. tuberosum* family 4-39-2-2 were of medium size for the species.

TABLE 10.—Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to leaf size

Family	Plants having leaves with an area of—												Total plants	Mean area of leaves ¹
	10 cm. ²	20 cm. ²	30 cm. ²	40 cm. ²	50 cm. ²	60 cm. ²	70 cm. ²	80 cm. ²	10 cm. ²	100 cm. ²	110 cm. ²	120 cm. ²		
<i>Solanum demissum</i>	No. 26	---	---	---	---	---	---	---	---	---	---	---	No. 26	6.4±0.5
<i>S. tuberosum</i> 4-39-2-2.....	---	3	9	7	1	---	---	---	---	---	---	---	20	28.5±4.0
<i>F</i> ₁	---	---	---	---	---	---	2	2	3	2	2	2	13	45.1±2.0
<i>F</i> ₂ 12.....	5	8	11	8	7	5	2	1	1	---	---	---	48	37.7±2.9
<i>F</i> ₂ 13.....	10	8	5	4	9	2	1	1	---	---	---	---	40	32.2±3.1

¹ Standard error is used rather than probable error.

Several stem characters were measured. The parents differed markedly in height, and, while each parent family varied considerably in stem length, there was no overlapping on a frequency distribution. The F_2 families showed a range in height from next to the shortest plants of *Solanum demissum* to approximately the mean height of the *S. tuberosum* parent, as shown in table 11. In this character, and also in internode length, shown in table 12, the F_2 families tended to be intermediate between the two parents, and were slightly shorter than the F_1 plants. In stem diameter at ground level, shown in table 13, the means of the F_2 families were intermediate between those of the parents and less than the mean of the F_1 . More than half of the F_2 plants resembled the *S. tuberosum* parent in having short stolons, but there were some plants in each family that resembled *S. demissum* in having long stolons, as is shown in table 14.

TABLE 11.—Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to stem height

Family	Plants having a stem height of—													Total plants	Mean stem height ¹
	2 inches	4 inches	6 inches	8 inches	10 inches	12 inches	14 inches	16 inches	18 inches	20 inches	22 inches	24 inches	26 inches		
<i>Solanum demissum</i>	No. 1													No. 27	In. 6.5±0.4
<i>S. tuberosum</i> 4-39-2-2.....		2	11	10	3		2	2	1	5	3	3	2	No. 21	18.7±1.0
F_1			1	3	6	4	1							No. 15	9.5±.5
F_2 12.....		2	8	11	13	8	2	3	1	1				No. 49	9.2±.5
F_2 13.....		5	10	13	4	4	3	1	1					No. 41	8.1±.5

¹ Standard error is used rather than probable error.

TABLE 12.—Internode length of the F_2 families compared with internode length of the parent families and a group of F_1 plants

Family	Plants measured	Mean internode length ¹	Family	Plants measured	Mean internode length ¹
	Number	Mm.		Number	Mm.
<i>Solanum demissum</i>	26	6.3±0.4	F_2 12.....	49	14.3±.9
<i>S. tuberosum</i> 4-39-2-2.....	21	28.1±1.2	F_2 13.....	41	12.4±1.0
F_1	15	19.1±1.2			

¹ Standard error is used rather than probable error.TABLE 13.—Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to stem diameter

Family	Plants having stems with a diameter of—																Total plants	Mean stem diameter ¹
	2.5 mm.	3.0 mm.	3.5 mm.	4.0 mm.	4.5 mm.	5.0 mm.	5.5 mm.	6.0 mm.	6.5 mm.	7.0 mm.	7.5 mm.	8.0 mm.	8.5 mm.	9.0 mm.	9.5 mm.	10.0 mm.		
<i>Solanum demissum</i>	No. 1	No. 3	No. 1	No. 10	No. 5	No. 6	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No. 26	Mm. 3.9±0.1
<i>S. tuberosum</i> 4-39-2-2.....					1	1	2		1	1		5	4	2	3	1	21	7.5±.3
F_1								2	2	2	1	5	5	3			15	7.4±.3
F_2 12.....	1		2	2	2	4	3	3	6	2	3	1	7	1		1	50	6.5±.3
F_2 13.....	4			5	4	7	6	4	1	2	1	5	2	3			44	5.4±.3

¹ Standard error is used rather than probable error.TABLE 14.—Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to stolon length

Family	Plants having stolon length of—												Total plants	Mean stolon length ¹
	2 cm.	4 cm.	6 cm.	8 cm.	10 cm.	12 cm.	14 cm.	16 cm.	18 cm.	20 cm.	22 cm.			
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	Centi- meters	
<i>Solanum demissum</i>													18	
<i>S. tuberosum</i> 4-39-2-2.....	13	3	4										20	
<i>F</i> ₁													15	
<i>F</i> ₂ 12.....	10	10	4	3	2	3	2	3		2	2		41	
<i>F</i> ₂ 13.....	14	10	5	2	3	1	1		1	1	1		39	

¹ Standard error is used rather than probable error.

The F_2 families set fewer tubers than their cultivated parent and far fewer than the F_1 plants, as shown in table 15. They also showed less variation in number of tubers set than did the cultivated parent. Both F_2 families and the cultivated parent showed great variation in yield. The total weight of all tubers of plants which set a crop ranged from 1 to 420 grams per plant, as shown in table 16, with the mean yields of the F_2 families nearly as great as the mean yield of the cultivated parent. Segregation for size of tuber was quite different from segregation for total yield, as may be seen by comparing table 17 with table 16. While none of the tubers of *Solanum demissum* weighed over 2 grams, and only 1 plant of the *S. tuberosum* parent produced a

tuber weighing more than 20 grams, 8 of the 14 F_1 plants, and 22 of the 78 F_2 plants produced tubers weighing more than 20 grams.

TABLE 15.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to the number of tubers set*

Family	Plants having indicated number of tubers											Total plants	Mean number of tubers ¹	Standard Deviation
	3	8	13	18	23	28	33	38	43	48	Over 50			
<i>Solanum demissum</i>	No. 11	No. 5	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1	No. 18	No. 7±3	8
<i>S. tuberosum</i> 4-39-2-2.....	7	1	1	3	1	1	4	1	1	2	2	24	24±5	20
F_1	13	7	6	6	4	1	4	2	1	3	3	15	40±4	-----
F_2 12.....	13	7	6	6	5	2	2	2	2	2	2	43	14±2	10
F_2 13.....	8	12	4	4	2	4	1	2	2	2	2	39	16±2	13

¹ Standard error is used rather than probable error.

TABLE 16.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to yield*

Family	Plants producing a crop that weighed—													Total plants	Mean crop weight ¹
	20 gm.	50 gm.	80 gm.	110 gm.	140 gm.	170 gm.	200 gm.	230 gm.	260 gm.	290 gm.	320 gm.	350 gm.	Over 365 gm.		
<i>Solanum tuberosum</i> 4-39-2-2.....	No. 11	No. 4	No. 1	No. 3	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1	No. 1	No. 24	Grams 86±22
F_1	14	9	6	5	1	2	3	2	2	2	1	1	1	14	238±16
F_2 12.....	14	9	6	5	1	1	1	1	1	2	1	1	2	42	85±16
F_2 13.....	12	9	6	3	1	1	1	1	1	1	1	1	1	34	58±11

¹ Standard error is used rather than probable error.

TABLE 17.—*Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to weight of largest tuber*

Family	Plants the largest tuber of which weighed within the indicated class limits													Total plants	Mean tuber weight ¹
	3 gm.	8 gm.	13 gm.	18 gm.	23 gm.	28 gm.	33 gm.	38 gm.	43 gm.	48 gm.	53 gm.	Over 55 gm.			
<i>Solanum demissum</i>	No. 26	No. 10	No. 6	No. 1	No. 6	No. 4	No. 4	No. 4	No. 4	No. 4	No. 4	No. 4	No. 1	No. 26	Grams 1
<i>S. tuberosum</i> 4-39-2-2.....	10	6	1	6	4	4	4	4	4	4	4	4	1	24	11±3
<i>F</i> ₁	14	4	3	5	4	4	4	4	4	4	4	4	1	14	21±1
<i>F</i> ₂ 12.....	14	4	3	8	4	4	3	3	3	1	2	1	1	43	18±2
<i>F</i> ₂ 13.....	11	9	4	3	2	2	2	3	3	3	1	1	1	35	13±3

¹ Standard error is used rather than probable error.

PHYSIOLOGICAL CHARACTERS

Such physiological characters as heat injury, frost injury, and maturity are of great importance to the potato grower. Because direct measurements of these characters are difficult, arbitrary scales were used.

The season of 1936 was extremely hot and dry, affording an excellent opportunity to study heat and drought injury. Among the F_2 plants it ranged from none to lethal injury, as shown in table 18. The

means were closer to the mean of the *tuberosum* than the *demissum* parent. The later renewal of growth by means of underground stolons, characteristic of *Solanum demissum*, was not found among the F_2 plants. Immaturity was recorded on September 27 on a scale of 0 to 5. At this time all plants of *S. demissum* and all the F_1 plants were still green, while all but two of the plants of the *S. tuberosum* parent were mature. The F_2 families showed a complete range from mature to green, as shown in table 19, the means falling halfway between the parent means. Frost-injury notes were taken on October 1 only on plants that were quite immature. Plants of *S. demissum* showed no injury from the light frost, while the two plants of the *S. tuberosum* parent that were still green were killed. Plants of the F_2 families showed a complete range from no injury to lethal injury, as shown in table 20, with 5 of the 54 plants on which frost notes were taken showing no injury. These five plants were too late in maturing to be of much use in northern latitudes, but it is probable that some of the F_2 plants which had matured before the frost struck possessed as much frost hardiness as these five plants.

TABLE 18.—Distribution of individuals of the parent and F_2 families and a group of F_2 plants according to relative heat and drought injury

Family	Plants showing indicated amount of injury				Total plants	Mean ¹ injury
	None, 0	Medium, 1	Severe, 2	Dead, 3		
	Number	Number	Number	Number	Number	
<i>Solanum demissum</i>	12	9	17	5	31	1.87±0.12
<i>S. tuberosum</i> 4-39-2-2.....	12	9	1	6	28	1.04±.22
F_1	9	31	10	6	15	.00±.00
F_2 12.....	17	17	10	9	56	1.23±.11
F_2 13.....					53	1.21±.15

¹ Arbitrary scale. Standard error is used rather than probable error.

TABLE 19.—Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to immaturity on September 27

Family	Plants showing immaturity ¹						Total plants	Mean ² immaturity
	0	1	2	3	4	5		
	Number	Number	Number	Number	Number	Number	Number	
<i>Solanum demissum</i>	20			2		26	26	5.0±0.0
<i>S. tuberosum</i> 4-39-2-2.....							22	.3±.2
F_1	12	9	5	5	7	11	15	5.0±.0
F_2 12.....	12	7	6	4	9	7	49	2.4±.3
F_2 13.....							45	2.3±.3

¹ On a scale of 0 for dead to 5 for all green.

² Arbitrary scale. Standard error is used rather than probable error.

Leaflets of the F_2 plants were shaped more like those of *Solanum tuberosum* than were the leaflets of the F_1 plants. Because of the greater vigor of the F_1 plants, their leaflets were longer and wider, and the mature leaf area was larger than in the F_2 plants. In stem height and diameter the F_2 plants were slightly smaller and more like *S. demissum* than the F_1 plants, and in internode length the F_2 were

definitely shorter and more like *S. demissum* than the F_1 plants. Stolon length was shorter and more like *S. tuberosum* in the F_2 than in the F_1 plants. Tubers of the F_2 were smaller and far fewer in number and the yield was far less than in the F_1 plants. In physiological characters the F_2 plants showed some heat injury whereas the F_1 did not, the F_2 were much earlier in maturing, and the F_2 plants were not quite as frost-hardy as the F_1 .

TABLE 20.—Distribution of individuals of the parent and F_2 families and a group of F_1 plants according to frost injury

Family	Plants showing injury rated as 1—						Total plants	Mean injury
	0	1	2	3	4	5		
<i>Solanum demissum</i>	Number 26	Number	Number	Number	Number	Number	Number 26	0 \pm 0.0
<i>S. tuberosum</i> 4-39-2-2.....						2	2	5.0 \pm .0
F_1	3	4	3	3	1	1	15	1.9 \pm .4
F_1 12.....	3	3	6	4	7	5	28	2.9 \pm .3
F_2 13.....	2	3	1	6	3	11	26	3.5 \pm .3

¹ On a scale of 0 to 5. (5=lethal injury).

² Arbitrary scale. Standard error is used rather than probable error.

BACKCROSS FAMILIES

Four of the F_1 plants were backcrossed to *Solanum tuberosum*, with plants 3, 5, 7, and 10 as the pistillate parents, and Minnesota selection 40-4-2-6, a fourth-generation inbred selection of the U. S. D. A. 38946, as the pollen parent. The F_2 families and the backcross families are compared in table 21. In leaflet shape, stem diameter, stolon length, number of tubers, immaturity, and frost injury, means of both F_2 families and of all four backcross families were intermediate between the means of the parents. In length, width, and shape of the largest simple leaf, means of the F_2 and backcross families were approximately equal. In cotyledon length, in which the parents were very nearly equal, means of the F_2 families and three of the four backcross families were smaller than the means of the parents. In size of largest tuber, the means of the F_2 families and the backcross families were approximately equal, surpassing both parents. The yield of the backcrosses were slightly greater than the F_2 families. In leaflet length and width and leaf area, the means of the F_2 families were larger than the means of the backcross families and larger than those of either original parent. The F_2 families were less injured by heat than the backcross families and were more similar to *S. tuberosum* families in this respect. They were more like *S. demissum* than the backcross families in cotyledon width and shape index, stem height, and internode length.

DISCUSSION AND CONCLUSIONS

Of the 15 F_1 plants grown from the cross between *Solanum demissum* and *S. tuberosum*, 1 set fruit with viable seed in the greenhouse but produced no tubers. The breeding behavior of this plant in the F_2 and F_3 generations suggests that it was not a hybrid but a specimen of *S. demissum*.

TABLE 21.—Characteristics of the four backcross families as compared with those of *E.* families 12 and 13

Families	Plants	Morphological characters									
		Cotyledon			Simple leaf			Leaflet			Mean leaf area
		Mean width	Mean length	Mean shape index	Mean width	Mean length	Mean shape index	Mean width	Mean length	Mean shape index	
<i>E.</i> 12	Number	Millimeters	Millimeters		Millimeters	Millimeters		Millimeters	Millimeters		Square centimeters
<i>E.</i> 13	56	4.4±0.2	7.5±0.3	0.60±0.02	23.1±0.6	30.8±0.7	0.75±0.01	32±1	45±2	0.70±0.01	38±3
Backcross:	50	4.0±.2	7.1±.4	.61±.02	19.1±.7	26.7±.8	.72±.01	28±2	40±2	.69±.01	32±3
41	40	5.0±.2	8.3±.3	.61±.02	19.1±1.0	25.2±1.3	.76±.01	28±2	38±2	.71±.02	32±2
42	15	4.4±.3	6.7±.5	.70±.03	19.8±1.5	25.7±2.0	.76±.02	19±1	31±2	.61±.03	29±3
43	70	4.8±.1	6.9±.2	.70±.02	20.4±.5	26.9±.7	.77±.01	22±1	33±1	.68±.02	32±2
	50	4.5±.2	6.4±.2	.73±.02	19.4±.8	26.5±1.1	.73±.01	20±2	31±2	.63±.02	25±2

Families	Plants	Morphological characters									
		Stem			Mean internode length	Mean stolon length	Mean number of tubers	Mean weight of largest tuber	Mean yield	Mean heat injury ¹	Mean imminuturity ²
		Mean height	Mean diameter	Mean shape index							
<i>E.</i> 12	Number	Inches	Millimeters		Centimeters			Grams	Grams		
<i>E.</i> 13	56	9.2±0.5	6.5±0.3	14±1	7.4±1.1	14±2	18±2	85±16	1.2±0.1	2.4±0.3	2.9±0.3
Back cross:	50	8.1±.5	5.4±.3	12±1	5.9±1.1	16±2	13±3	58±11	1.2±.1	2.3±.3	3.5±.3
40	40	12.1±.6	5.6±.3	18±1	9.8±1.4	14±2	19±4	82±18	1.4±.1	2.3±.3	4.4±.2
41	15	13.6±1.3	5.2±.4	21±2	4.6±1.4	14±4	13±3	60±21	1.0±.2	2.3±.6	4.6±.2
42	70	11.7±.6	4.9±.2	18±1	9.0±1.0	21±3	19±2	123±18	1.5±.1	3.1±.2	3.6±.2
43	50	11.6±.7	4.9±.2	19±1	7.4±.9	14±2	21±6	35±16	1.4±.1	3.5±.3	3.3±.2

¹ Standard error is used rather than probable error.² Arbitrary scale from 0 to 3.³ Arbitrary scale from 0 to 5.

The remaining 14 plants appeared to be true hybrids, and chromosome counts in some of them showed the presence of the expected 60 chromosomes. In the greenhouse they flowered but did not set selfed seed, though they set moderately well when backcrossed to *Solanum tuberosum*. The presence of a sufficient supply of viable pollen for self fertilization was indicated by pollen stainability of 24 to 45 percent in the greenhouse, and over 50 percent at Castle Danger. Since the work of Krantz et al. (9a) has shown that pollen that is 20 to 50 percent stainable cause sseed production in 76 to 94 percent of the plants of *S. tuberosum*, it was assumed that because of the proportion of stainable pollen found in the F₁ plants, some of them would produce seed. Despite this moderate amount of stainable pollen, viability on the agar medium used was only 2 percent, but even this low proportion of functional pollen among the many thousands of grains in each flower would insure a sufficient supply for seed production.

Artificial self-pollination in the greenhouse and in the field at University Farm failed to produce fruit setting. Three of the F₁ plants were moved to an environment more favorable to seed production, where they were highly vegetative and bloomed profusely throughout the summer. Of the three, plant 12 set a moderate amount of fruit and had the most seeds in each fruit. Plant 13 had fewer fruits, less seed in each fruit, and a lower rate of germination of seed. Plant 14, which bloomed the most profusely, set very few fruits, and the few seeds obtained failed to germinate.

Chromosome counts in 15 plants of F₂ family 12 were lower than would be expected if 48 of the 60 chromosomes in the F₁ plants paired into bivalents and the 12 chromosomes of the fifth genom were distributed at random. Ovules and pollen grains would then contain between 24 and 36 chromosomes each, and if all gametes were equally viable, the counts in the F₂ would range from 48 to 72, with a mean of 60. Irregularities seen in pollen formation indicate that some of the chromosomes were lost in meiosis, and the low chromosome counts in the F₂ plants could be explained wholly on this basis. A more probable explanation is the fertilization of ovules containing 24 to 36 chromosomes with pollen containing 24 chromosomes. Since in many genera pollen with extra chromosomes is not functional, it is possible that only the 24-chromosome pollen of the F₁ plants was functional. Chromosome counts of 48 to 60 could thus be obtained by self pollination of the F₁ plants, as well as by backcrossing the F₁ plants with the 24-chromosome pollen of *Solanum tuberosum*.

Segregation in the progenies of plants 12 and 13 showed a range from the extremes of *Solanum demissum* to the extremes of *S. tuberosum* selection 4-39-2-2, though most of the F₂ plants were more like *tuberosum* than were the F₁ plants. Both F₂ families 12 and 13 had larger leaves, larger terminal leaflets, and larger tubers than those of either parent.

The data show that ovules of the F₁ plants are moderately fertile but that the pollen is sparingly viable, and self-fertilization of the F₁ is either difficult or impossible. The F₂ families obtained do not differ markedly from the backcross families, and it appears that the naturally set seed of F₁ plants 12 and 13 may have been the result of accidental backcrossing with *Solanum tuberosum*.

SUMMARY

Inheritance of some characters in the interspecific cross *Solanum demissum* Lindl. \times *S. tuberosum* L., was studied.

Solanum demissum is a small, prostrate, late-maturing species with narrow cotyledons, small leaves, long stolons, and many small tubers. It has been reported to have 72 chromosomes. *S. tuberosum* selection 4-39-2-2, the pollen parent is a large, erect, early-maturing, frost-tender selection with wide cotyledons, medium-sized leaves, short stolons, and many medium-sized tubers. *S. tuberosum* has been reported to have 48 chromosomes.

The 60-chromosome hybrids between those species showed considerable irregularity in meiosis, nonoriented and lagging chromosomes being usual. Fifteen plants were raised, of which three were fertile enough to produce F_2 families.

One F_2 family was practically identical with *Solanum demissum* in morphological characters and was uniform.

The other two F_2 families approached *Solanum tuberosum* in morphological characters and were variable. Chromosome counts in 15 of the plants of one of these families ranged from 48, the number in *S. tuberosum*, to 58, or 2 less than the number in the F_1 plants.

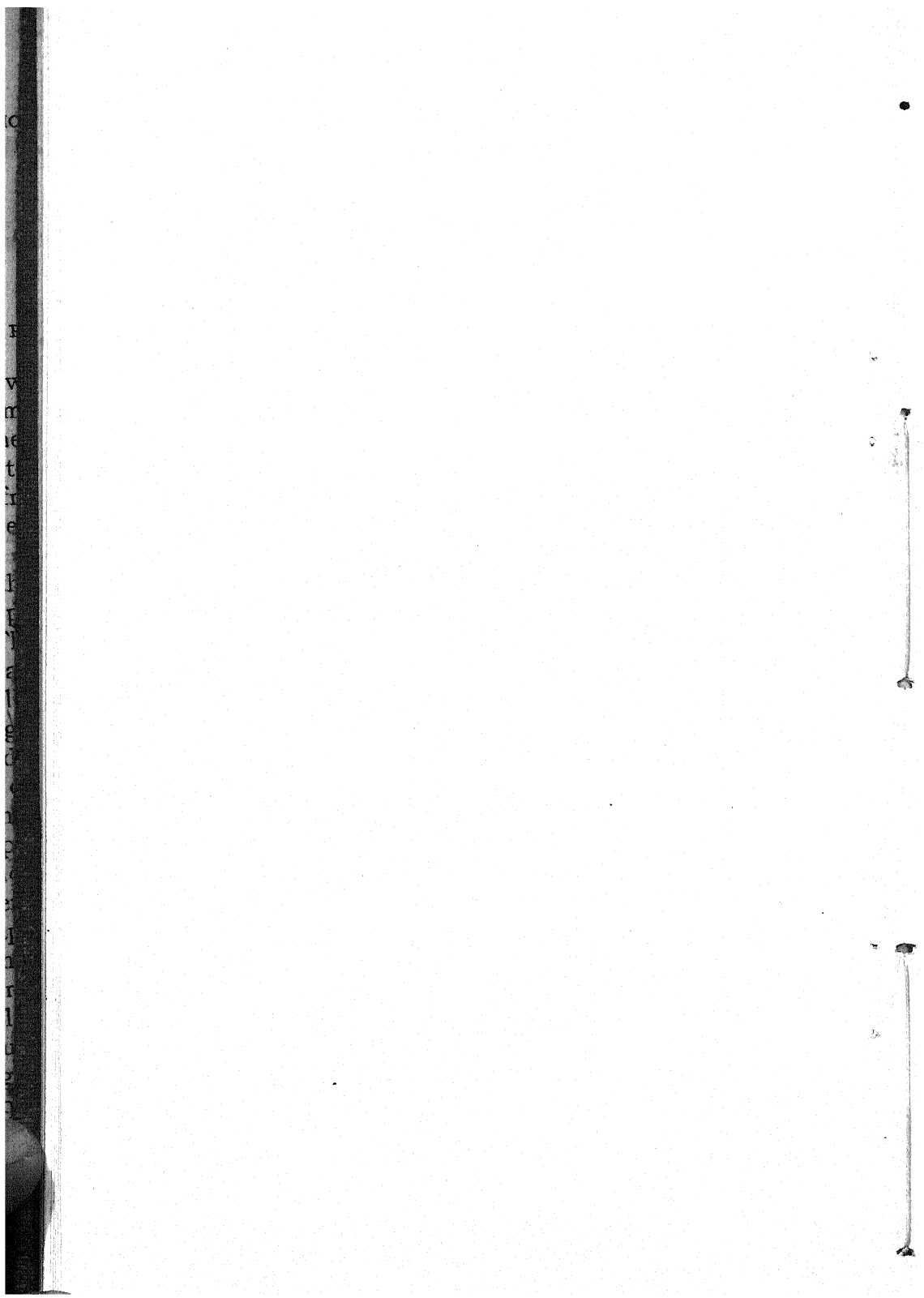
In cotyledon measurements, stem diameter, heat injury, maturity, frost injury, stolon length, number of tubers, and weight of the largest tuber, these F_2 families showed a range from the extreme of one original parent to the extreme of the other, with the means falling halfway between the parent means. Height of mature plants and internode length of the F_2 families resembled *Solanum demissum* more than 4-39-2-2, while the weight of the crop was similar to that of the cultivated parent. Terminal leaflets and mature leaves were larger than those of either parent.

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EFFECT OF TEMPERATURE ON THE RATE OF DETERIORATION OF FRESH VEGETABLES¹

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INTRODUCTION

In recent years many improvements have been made in methods of handling fresh vegetables. Cold-storage rooms are being used extensively for long- and short-time storage, and shipments to distant markets are made in refrigerated cars or refrigerated trucks, the load frequently being precooled before it leaves the shipping point. All these practices are based on the well-recognized fact that the biological processes responsible for the break-down of the produce are greatly retarded by a lowering of the storage temperature. This fact is emphasized by the results of storage experiments carried out with many different vegetables. Generally, a lowering of the storage temperature has resulted in a considerable lengthening of the storage life of a particular vegetable, provided the temperature was not so low as to cause injury from freezing or chilling.

While nearly all of these experiments indicate a close relationship between temperature and the rate of deterioration, few data are available which show in numerical terms exactly what this relationship is. In some cases only one or two different storage temperatures were used, and usually only the temperature of the room, instead of that of the vegetable, was recorded; no account being taken of the fact that many hours, sometimes days, elapse before the temperature of vegetables in a commercial package reaches equilibrium with the temperature of the surrounding atmosphere. While such experiments may solve specific, practical problems, they are of little value in establishing broad, general principles governing the relationship between temperature and storage life of different vegetables. If it were possible to deduce a general rule which would express this relationship with a reasonable degree of accuracy, such information should furnish a valuable basis for solving many practical problems involved in the storage and transportation of fresh vegetables.

Several difficulties present themselves in determining the relationship between temperature and rate of deterioration. Different factors, such as respiration, transpiration, translocation, and metabolic activity, may contribute to the break-down of vegetables in storage. In some vegetables, such as sweet corn and peas, a pronounced lowering of palatability results from the loss of sugar. Usually this process occurs at a rate which is much more rapid than wilting or other changes in the external appearance would indicate. Furthermore, the rate of sugar loss in vegetables is by no means uniform. Appleman and Arthur (1),² working with sweet corn, and Bisson, Jones, and Robbins

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² Italic numbers in parentheses refer to Literature Cited, p. 58.

(4), in experiments with asparagus, showed that these vegetables, when held at a high temperature, lose sugar very rapidly at first. Later, after most of the sugar has been depleted the rate of loss is greatly reduced; in fact, the percentage of sugar may actually increase toward the end of the storage period as a result of hydrolysis of other carbohydrates.

In many leafy vegetables, the first symptom of deterioration is an excessive wilting of the tender tissues. This may or may not be associated with observed changes in chemical composition. In storage experiments with snap beans, Parker and Stuart (8) showed that the percentage composition changed only very little, even though some lots were held for several days at room temperature. Ultimate deterioration of snap beans is usually brought about by shriveling and by decay through the spread of disease organisms.

From these considerations, it becomes evident that the results of any study of the rate of deterioration will depend largely on the criterion employed in measuring the rate of break-down. Nevertheless, when different lots of vegetables have reached a point where they become entirely unsalable, their appearance is very nearly alike regardless of the temperature at which they were kept. Chlorophyll-bearing parts of the plant tissue begin to turn yellow, many of the plant cells have died, or collapsed, or reached the stage of permanent wilting; secondary infection with bacteria and fungi begins to spread rapidly. On approaching this condition a salable product may change into a decaying mass with surprising speed, sometimes within a few hours.

In spite of the difficulties outlined, it was felt worth while to carry out a study to determine the relation between temperature and rate of deterioration provided proper precautions were taken to obtain comparable data and provided the limitations of such experiments were fully recognized.

EXPERIMENTAL METHODS

All vegetables used in these experiments were either grown on nearby truck farms or in the gardens of the Department of Vegetable Crops at Cornell University, Ithaca, N. Y. In every case the experiments were started within 4 hours after the vegetables had been harvested. Only produce of good quality, free from disease injury, was used in these studies.

The storage experiments were carried out in the experimental cold-storage rooms of the Department of Vegetable Crops. Four rooms were used in which the temperature was held at 35°, 50°, 65°, and 80° F., respectively. The corresponding relative humidity in these rooms averaged 94, 82, 74, and 68 percent. Temperature fluctuations were less than 3° and the relative humidity varied not more than 5 percent. No attempt was made to maintain the same relative humidity in all the rooms since this would have presented considerable technical difficulty, and also because under conditions of commercial practice, the prevailing relative humidity is nearly always higher at the lower storage temperature, and vice versa.

The vegetables used in the experiments were peas (*Pisum sativum* L.), spinach (*Spinacia oleracea* L.), radish (*Raphanus sativus* L.), lettuce (*Lactuca sativa* L.), celery (*Apium graveolens* L.), brussels sprouts (*Brassica oleracea* var. *gemmifera* DC.), asparagus (*Asparagus officinalis* L.), and sweet corn (*Zea mays* var. *rugosa* Bonafous).

The vegetables were stored in commercial containers, that is, peas and spinach in 1-bushel baskets, radishes in one-half-bushel baskets, lettuce in crates containing 2 dozen heads, celery in two-thirds celery crates, sweet corn in bags containing 5 dozen ears, brussels sprouts in 1-quart baskets, and asparagus in 2-pound bunches. Duplicate lots were used for all vegetables.

Accurate temperature records of each lot were kept by placing two or three thermometers near the center of each container. Depending on the rate of temperature change within the containers, readings were taken every hour, every 2 or 3 hours, or twice daily. From these records, curves were drawn showing accurately the temperature changes in each lot.

At frequent intervals each lot was inspected for condition and careful notes were taken on the progress of deterioration. A special effort was made to determine as accurately as possible the time at which each lot became unsalable. With some practice it became possible to estimate the time required to reach this stage of break-down with sufficient accuracy so that two workers making observations independently would obtain results varying less than 7 percent of the total storage time.

For most vegetables the relative rate of break-down of different lots was determined by comparing the time required to render these lots totally unsalable. As was pointed out before, the palatability of some vegetables, particularly those with a high sugar content, is subject to a more rapid deterioration than their appearance would indicate. For that reason the rate of deterioration of sweet corn, peas, and asparagus was also determined by taking samples for chemical analysis at certain intervals. Analyses for sugars were made by standard methods, as described in methods of the Association of Official Agricultural Chemists (3).

METHOD OF CALCULATING RESULTS

The relative rate of break-down in vegetables at different temperatures may be expressed in two ways. Calculations may be based either on the temperature of the storage room or on the temperature of the vegetables themselves. As illustrated in figure 1, the temperature of a vegetable when stored in commercial containers may differ widely from that of the surrounding atmosphere. A lot of spinach, for instance, which had an initial temperature of 90° F. and was held at 35° did not reach temperature equilibrium until the end of 60 hours. Obviously the rate of deterioration was much more rapid during the first 60 hours than later. Correspondingly, the average temperature of another lot held at 80° was about 14° higher than that of the surrounding air as a result of heat evolved during rapid respiration. Consequently, entirely different results may be obtained depending on whether the temperature of the storage room or that of the vegetable itself is used as a basis for calculating the temperature coefficient.

Considering first the temperature of the storage room only, the experimental results were expressed in a series of curves (fig. 2) which indicate for each vegetable how long they can be held at the different storage temperatures before complete break-down occurs. Although these curves do not express the true relationship between temperature and rate of deterioration, they may become valuable in solving certain practical storage problems.

In order to establish the true relationship between temperature and the rate of break-down the temperature of the vegetable itself, rather than that of the room, had to be considered. First, it was necessary

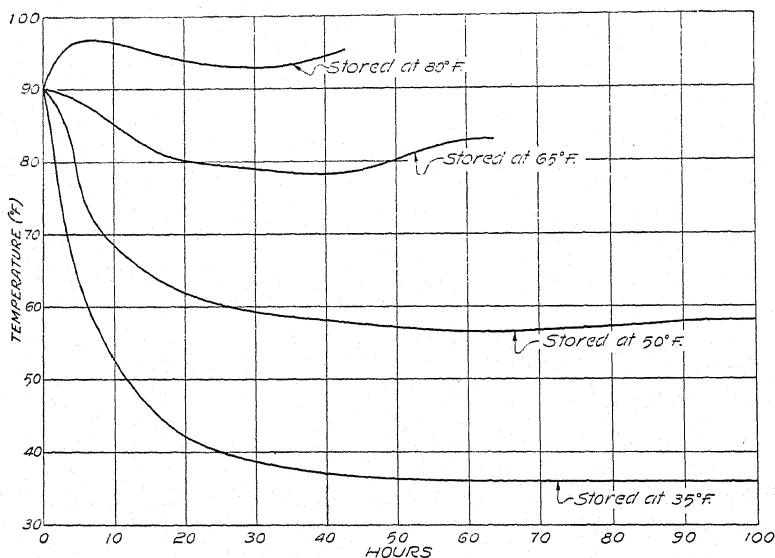


FIGURE 1.—Temperature inside the containers of four lots of spinach after transfer from 90° F. to rooms held at a constant temperature of 35°, 50°, 65°, and 80° respectively.

to calculate for each experimental lot a temperature coefficient which would express the rate of break-down at that particular temperature. The coefficient corresponding to a temperature of 32° F. was arbitrary

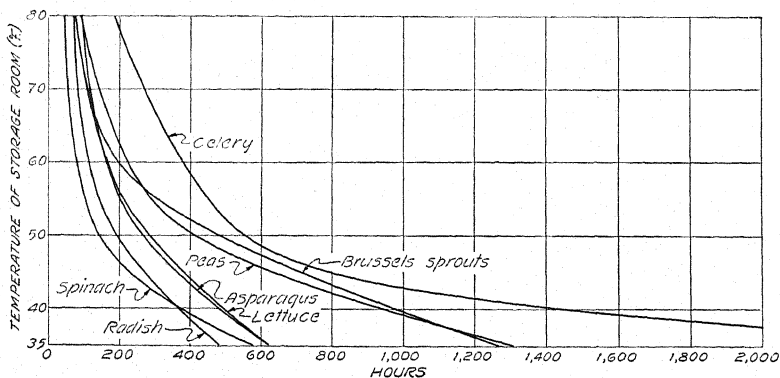


FIGURE 2.—Time-temperature curves based on the temperature of the storage rooms, indicating how long different vegetables can be held at a definite room temperature before complete deterioration occurs.

trarily set at 1. The coefficient for any other temperature then is a rate factor which indicates how much more rapidly deterioration occurs at that temperature than it does at 32°. Provided the temperature of each experimental lot is known and remains constant

throughout the storage period, the coefficient for each lot can easily be calculated by dividing the total storage time of a vegetable held at 32°, usually expressed in hours, by the total storage time of the particular lot in question. Total storage time in this case is the period from harvest until the vegetables have reached a stage at which they become unsalable. Assuming for instance that the storage life of a vegetable is 300 hours at an average temperature of 32° and 100 hours at 50°, the temperature coefficient corresponding to 50° would be 3. It also follows that the total storage time multiplied by the corresponding temperature coefficient must give a product which is a constant for any one vegetable. This may be expressed in the form of an equation $C_t \times h = K$, where C is the temperature coefficient corresponding to the temperature t , h the total storage time at that temperature, and K a constant. Since it was postulated that at a temperature of 32° the value for C_t is equal to 1, the value for K must be numerically equal to the storage time h at that temperature.

Because the average temperature of the experimental lots was never as low as 32° F., the total storage time for that temperature and the value for K had to be found by extrapolation. The calculation of the coefficients was further complicated by the fact that the temperature of each lot fluctuated over a fairly wide range, even though the room temperature remained constant. In addition, an inspection of the data indicated that the rate of deterioration increases geometrically with a rise in temperature. Obviously, a serious error would be introduced if one were to use the arithmetic mean of each temperature curve in these calculations, since too little weight would be given to the higher temperature values of the curves.

No simple method was available by which the temperature coefficient could be calculated directly from the available data because of the complications mentioned. It was possible, however, to arrive at fairly accurate values by a method which in principle consists of making a series of approximations to find a curve which gives the correct coefficients for each vegetable at different temperatures. All calculations were based on the supposition made earlier that for every lot of vegetables the product $C_t \times h$ must be the same and equal a constant K . The correctness of each curve could then be tested by inserting values for time and temperature as determined experimentally. Theoretically the curve would be perfect if the product $C_t \times h$ would be exactly the same for each one of the four lots of the same vegetable. How these calculations were carried out in detail can best be explained by using an example.

Figure 1 shows the temperature curves for four lots of spinach held at 35°, 50°, 65°, and 80° F., respectively. The total storage time required to render each lot unsalable is given in table 1.

TABLE 1.—Rate of deterioration in spinach at four different storage temperatures

Item	Data for room temperature of—			
	35° F.	50° F.	65° F.	80° F.
Total storage life, hours.....	576	141	64	43
Rate ratio.....	1	4.09	9.00	13.40
Values for K as calculated from curve b^1	748.8	536.8	643.0	741.1
Values for K as calculated from curve c^1	845.9	842.7	840.4	849.1

¹ Of figure 3.

In order to establish a preliminary rate curve which could later be corrected by the method of approximations it was necessary to disregard temporarily the error which results from basing calculations on the arithmetic mean of the temperature curves. Thus the arithmetic means of the temperature curves for the 35° and 80° F. lots were determined to be about 38° and 94.5° while the corresponding storage periods for these two lots were 43 and 576 hours, respectively. In other words, the rate of break-down of the first lot was $\frac{576}{43}$ or 13.4 times more rapid than that of the second one. Assuming that the rate increment between these two temperatures is a simple logarithmic function, approximate values for the coefficients in the intermediate temperature range could be found by the graphic method. The temperature was plotted on the abscissa on an algebraic scale and the

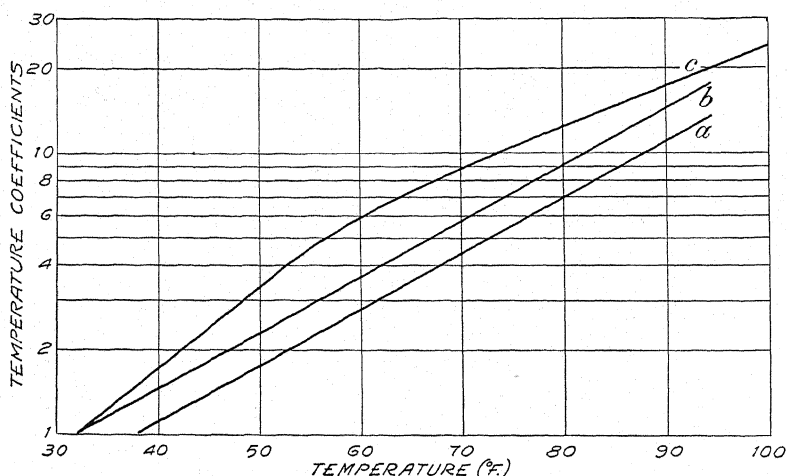


FIGURE 3.—Preliminary and final temperature coefficients for spinach as determined by a method of approximations. See text for explanation.

corresponding coefficients on a logarithmic scale (fig. 3, *a*). Assuming that the decrement between 36° and 32° continues at the same rate, a second line, *b*, could be drawn parallel to line *a* in such a way that line *b* intersects the base line at 32°. Thus it was possible to obtain temperature coefficients the numerical values of which were based on a coefficient of 1 for 32°, and it was also possible to obtain values for the constant *K* which would show in comparable numerical terms how much longer one kind of vegetable can be held than another at that temperature.

Next it was necessary to eliminate the error which was introduced when the arithmetic rather than the logarithmic mean of the temperature curves (fig. 1) was used in calculating the coefficients. This was done by determining separately for each 5-hour interval the average temperature and the corresponding approximate coefficient, using the values given by curve *b*, figure 3. Next, each coefficient was multiplied by 5 and the sum of these products was calculated separately for each temperature curve, thus giving the values for $C_i \times h$ of each experimental lot. Provided the true values for C_i had been

found, the product $C_t \times h$ should be the same, and should equal a constant K for every lot of the same vegetable regardless of the temperature at which it was stored. Table 1 shows the values for K as calculated from the temperature coefficients represented by figure 3, *b*. While the calculated values of K for the lots stored at 32° and 80° agree fairly well they are much too low for those kept at 50° and 65°. In calculating temperature coefficients for other vegetables by the same method, it became apparent that the coefficients for the temperature range between 50° and 70° F. always gave too low values. This led to the conclusion that the increase in the rate of deterioration of vegetables is not uniformly logarithmic over the entire temperature range used in these experiments. Instead, when plotted on a semi-logarithmic scale as in figure 3, the true rate curve consists of two separate, fairly straight lines which intersect somewhere between 50° and 60°. Based on this observation, a third curve, *c*, was drawn, the slope of which was steeper at the lower and flatter at the higher temperatures. The exact position of this curve was determined by a series of approximations. The slope of the two sections was changed until finally values for the temperature coefficients were obtained, which, in calculating the K values of the four experimental lots, gave results which were practically identical (table 1). These final values for K show less than 1-percent deviation from the mean, indicating that the temperature coefficients as represented by curve *c* (fig. 3) correspond accurately to the true rate of deterioration at the different temperatures.

It should be pointed out that while this method of calculating the temperature coefficients is indirect and one of trial and error, the final results obtained are nevertheless very accurate and the degree of error is expressed exactly by the deviations from the mean of the K values as calculated for different experimental lots. While the temperature coefficients were calculated from the experimental data of four different lots only, the results are actually based on more than 50 single observations, since the average temperature for each 5-hour interval was considered in determining the exact position of the coefficient curves. The only assumptions made are that the coefficient when plotted against the temperature must follow a single smooth curve; also that the rate decrement between 36° and 32° F. is about the same as in the range between 50° and 36°.

The coefficients of the other vegetables were calculated in the same manner. The process of arriving at the true values was repeated in every case until the deviations from the mean value of K was never greater than 3 percent.

This method of arriving at the temperature coefficients may be criticized as being unnecessarily cumbersome and the suggestion may be offered that the same results could have been obtained more easily by making provision for bringing the vegetables to a constant temperature very rapidly, thus avoiding the complications due to temperature fluctuations in the experimental lots. The only way this could be accomplished is by spreading the vegetables on the floor of the constant-temperature room. Preliminary experiments showed that by doing so a constant temperature was attained within a few hours and subsequent reheating of the vegetables was prevented effectively. On the other hand, it was noticed that these vegetables when spread

out were subject to excessive wilting. It was also found that the rate of deterioration and the symptoms of break-down under these conditions were somewhat different from those observed in lots stored in commercial containers. On the basis of these findings, it was felt that the method of experimentation actually used would yield data which are more useful in solving such practical problems as are met in connection with precooling, transportation, and temporary cold storage of vegetables.

RESULTS AND DISCUSSION

TEMPERATURE COEFFICIENTS

The temperature coefficients of seven different vegetables showing their relative rate of deterioration are given in figure 4. The temper-

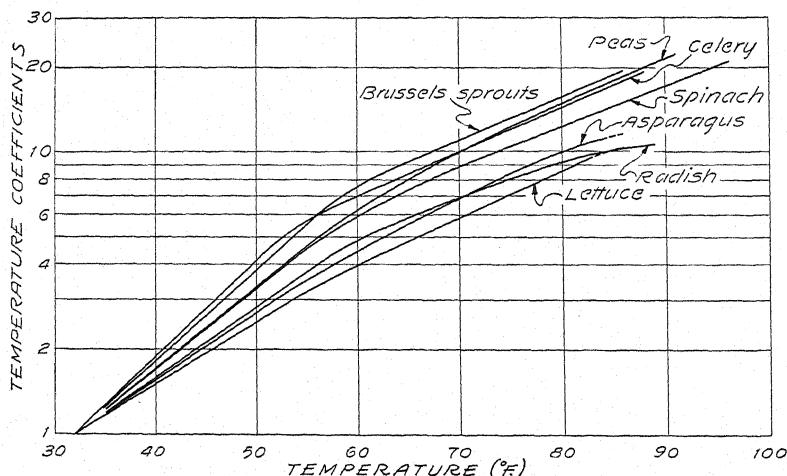


FIGURE 4.—Coefficients indicating the rate of deterioration of seven vegetables at different temperatures. Values are based on a coefficient of 1 for 32° F.

ature of the vegetable is plotted on the X axis on an algebraic scale, while the corresponding coefficients are plotted on the Y axis on a logarithmic scale. Each resulting curve follows two fairly straight lines which intersect between 50° and 65° F. In other words, the increment in the rate of deterioration is fairly uniform below 50° and again above 65°, the increment being more rapid in the lower than in the higher temperature range. The break in each one of the curves can be explained only by assuming that above 50° a new, important factor enters the biological reactions that determine the rate of break-down. Most likely this new factor is closely associated with the respiration occurring in the plant tissue. Other factors which may be responsible for the observed change in the reaction rate are the activity of various enzymes and the growth rate of micro-organisms at different temperatures. Many experiments have shown that below 77° the respiration rate of plant tissue increases logarithmically with a rise in temperature. Above that temperature the rate continues to increase, but much more slowly, until finally a point is reached beyond which the respiration rate declines rapidly to a very

low level. Thus Gerhart (5), studying the rate of respiration in strawberries, found that above 77° the increment in the respiration rate was less than below that temperature. Similarly, Gore (6) found that the increase in the respiration rate of various fruits was slightly reduced at higher temperatures. However, in both these studies the break in the respiration curve did not become very pronounced until a temperature of 90° was attained. Gerhart explains the break in the respiration curve by assuming that at high temperatures the permeability of the cell wall to gases becomes a limiting factor, causing a deficiency of oxygen and an accumulation of carbon dioxide and other end products in the plant tissue. Whether or not changes in the respiration rate are the principal cause for the observed break in the deterioration curves of vegetables remains questionable. Doubt may be cast on such an explanation in view of the fact that the break in these curves occurred at temperatures between 50° and 65° , while Gerhart, Gore, and others found that the respiration rate of fruits increased logarithmically until the temperature reached 77° or 95° . It must be kept in mind, however, that in the last-mentioned studies on respiration only a few isolated fruits were used. It is quite possible that even at a temperature of 55° the oxygen concentration within a commercial container becomes sufficiently low to cause an appreciable lowering of the normal respiration rate. This reduction in the respiration rate would not result under conditions where relatively little plant tissue is in contact with air of normal atmospheric composition as was the case where the respiration rate of fruits and vegetables was being studied experimentally.

It is interesting to compare the temperature coefficients of different vegetables for any one temperature. One might suspect that those vegetables that are usually considered as being highly perishable have also a high temperature coefficient. An inspection of figure 4, however, shows that such is not the case. On the contrary, brussels sprouts, peas, and celery, which resist deterioration in storage for a relatively long period of time, had the highest coefficients. In other words, a definite increase or decrease in the storage temperature affected the total storage life of these vegetables more than it did that of asparagus, radish, and lettuce, which are subject to comparatively rapid break-down even at 32° . While this unexpected relationship holds true fairly well in the seven vegetables studied, it would be necessary to carry out experiments with a large number of other vegetables to find out whether or not these observations can be stated as a general rule. It should be emphasized here that the temperature coefficients are not a measure of the actual rate of deterioration at any one temperature. Instead, they merely indicate the degree of acceleration of the deterioration process in comparison with the rate at which this process proceeds in the same vegetable at 32° . Celery, for instance, although it had relatively high temperature coefficients, never attained the same rate of break-down as some of the other vegetables even in the higher temperature range, simply because the basic rate of break-down at 32° was very much lower than it was in the more perishable vegetables.

RATE OF DETERIORATION AND VAN'T HOFF'S RULE

It has frequently been proved that most chemical and biochemical reactions *in vitro* follow Van't Hoff's rule fairly closely. This rule

states that the reaction rate increases two or three times with every 10° C. rise in temperature. It has also been shown that many physiological processes of micro-organisms and higher plants, such as growth and respiration, conform at least roughly to Van't Hoff's rule. Based on these observations this rule has been cited to illustrate the important relationship between temperature and the rate of deterioration of fruits and vegetables. Thus Kidd and West (7) showed that both the rate of break-down and the rate of respiration of apples in storage obey Van't Hoff's rule at temperatures ranging from -1° to $+18^{\circ}$. The rate factor indicating exactly how much the reaction rate increases with a 10° rise in temperature is usually expressed as Q_{10} .

From the temperature coefficients given in figure 4 it was easy to calculate the Q_{10} values of the seven vegetables at three temperature ranges of 10° C. each (table 2).

TABLE 2.— Q_{10} values for the 7 vegetables considered in Figure 4 at 3 different temperature ranges

Temperature range ($^{\circ}$ F.)	Celery	Brussels sprouts	Peas	Spinach	Radish	Lettuce	Asparagus
32-50	4.12	3.82	3.33	3.33	2.87	2.48	2.68
50-68	2.27	2.70	2.76	2.49	2.26	2.22	2.37
68-86	1.94	1.87	2.03	1.83	1.58	1.87	1.84

First of all it will be observed that at the lowest temperature range of 32° – 50° F. celery and brussels sprouts have the highest Q_{10} values. As the temperature is raised, the Q_{10} values decrease in all vegetables, more rapidly in those which at the lower temperature had relatively high-values, so that at the highest temperature range of 68° – 86° the Q_{10} values became fairly uniform for all vegetables, varying only from 1.58 to 2.03. The important conclusion derived from table 2 is the fact that the Q_{10} value expressing the increment in the rate of deterioration follows Van't Hoff's rule very roughly only. The calculated values not only vary with different vegetables, but they also change with different temperature ranges for the same vegetable. It is not permissible therefore to apply these rate values to temperature ranges other than those for which they were found experimentally.

TIME-TEMPERATURE CURVES

From the data presented in figure 4, it was possible to express the temperature relationship in the form of time-temperature curves (fig. 5) similar to those presented in figure 2. In both cases the data are plotted on the same scale; the only difference is that in figure 2 the maximum storage life of the vegetables is plotted against the temperature of the storage room, while in figure 5 it is plotted against the temperature of the vegetable itself. Consequently figure 2 is more useful in determining the possible storage life of vegetables held at a constant temperature, while the data presented in figure 5 should be helpful in studies where records are available of the temperature of the vegetables regardless of whether these temperatures remain constant or fluctuate over a wide range.

Regardless of the method used for calculating the time-temperature curves, the data show that a lowering of the temperature by a definite

number of degrees in the range above 55° F. has very little effect on the total storage life of the vegetables studied, while the same decrease in temperature below 55° causes a very appreciable lengthening of their life span. This, of course, is due to the fact that the Q_{10} values are much smaller in the upper than in the lower temperature range. These findings are contrary to the common belief that the increment in the deterioration rate becomes progressively greater as the temperature is raised.

Applying these findings to practical problems of storage and transportation, one must conclude that any precooling, icing, or refrigeration method used is of little benefit unless it is sufficiently effective to lower the temperature of the vegetables to 55° F. or lower. In other words, the effect of refrigeration in delaying ultimate break-down

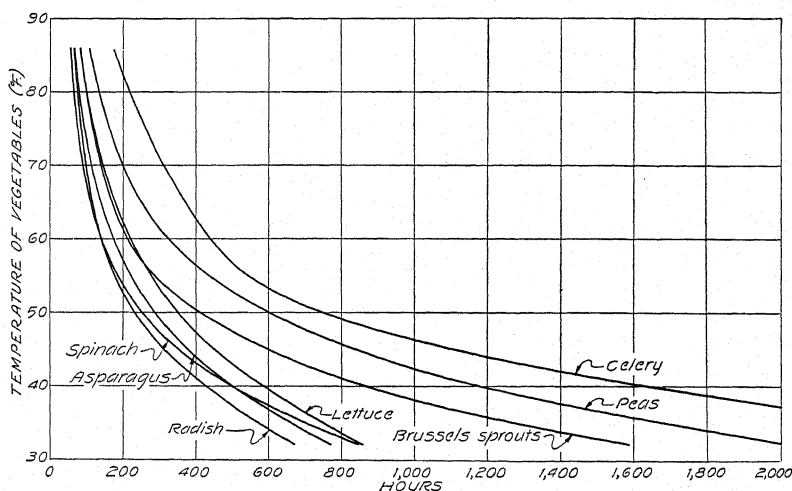


FIGURE 5.—Time-temperature curves showing the time required to bring about complete deterioration of seven different vegetables if the temperature of the vegetables is the same as indicated on the ordinate.

becomes increasingly greater as the lower temperature range is approached. A comparison of figures 2 and 5 shows the total storage life of the different vegetables at any one temperature to be somewhat longer when the data were based on the temperature of the vegetables rather than on the temperature of the room. This is to be expected because the actual average temperature of the vegetables is always higher than that of the storage rooms.

THE APPLICATION OF TEMPERATURE COEFFICIENTS AND TIME-TEMPERATURE CURVES TO PRACTICAL PROBLEMS

The data presented in figures 3 and 5 not only make it possible to predict how long a certain vegetable will keep, provided its temperature history is known, but they also permit the calculation of the relative effectiveness of different shipping or precooling methods. The procedure to be followed is similar to that used in calculating the temperature coefficients. First, it is necessary to find the temperature coefficients corresponding to the average temperature of

small intervals on the temperature curves. Next, each coefficient is multiplied by the respective number of hours of the time interval and the sum of these products is determined. The fraction that this sum is of the constant K of that particular vegetable will indicate exactly how far deterioration of the lot in question has proceeded. In this way it can be shown, for instance, that a lot of peas properly precooled and shipped from the West coast to New York City is likely to arrive in better condition than a similar lot which is shipped by truck from within New York State without precooling. Using hypothetical data, it was calculated that deterioration on arrival had advanced to the extent of 20 percent in the first and 35 percent in the second lot. This difference in conditions in favor of the peas from the West coast prevails in spite of the fact that this lot was in transit for 10 days, while the peas from New York State were assumed to have reached the market within 30 hours.

Here again it should be pointed out that any differences in the degree of deterioration as calculated from the temperature coefficients are not necessarily reflected in their external appearance. When an attempt was made to measure the rate of deterioration of different vegetables by means of a grading system, it was found that the first symptoms of break-down could not be recognized easily until one-fourth or one-third of the total deterioration process was completed. Also, it was noticed that the rate at which visible changes in appearance took place at any one temperature became progressively more rapid as the final stages of break-down were approached. In other words, the rate of visible break-down under definite conditions is not all uniform throughout the entire process. Consequently, the temperature coefficients apply strictly speaking only to the rate of the complete process; they cannot be used safely to determine how far visible deterioration may progress under certain conditions within a given time interval.

Needless to say, any calculations based on figures 1 to 5 can give results which are only approximately correct. While the condition of the vegetables used in these experiments was probably fairly representative of good commercial lots, there are many variables such as variety, growing conditions, and stage of maturity that may have a pronounced effect on the keeping quality of a certain vegetable. It seems reasonable to assume that although these variables may change the constant K to an appreciable extent, there is less likelihood that they will have much influence on the temperature constant of any one kind of vegetable.

EFFECT OF PREVIOUS COLD STORAGE ON THE RATE OF DETERIORATION AT HIGHER TEMPERATURES

In recent studies on the respiration of vegetables under various conditions, Appleman and Smith (2) found that the respiration rate of certain kinds is temporarily accelerated as a result of previous prolonged exposure to low temperatures. These findings would suggest that precooling or temporary cold storage may have a detrimental effect on the keeping quality of vegetables at subsequently higher temperatures. Such an assumption is in agreement with the belief held by many growers and shippers that vegetables after precooling tend to deteriorate more rapidly than other lots which have not been

precooled. Obviously, if the contention were correct that the previous temperature history of a vegetable has a direct effect on the subsequent rate of break-down the results of this investigation would be of doubtful value, except in those cases where the temperature remained fairly constant throughout the storage period.

A series of experiments was carried out by the writer in which several vegetables were held at 35° F. for periods ranging from 1 day to several weeks and then were transferred to higher temperatures. The results of these studies will be described in a separate paper. It suffices to say that in no case did the initial low temperature have any noticeable effect on the rate of break-down at higher temperatures. In general, where the vegetables had been held at 35° for more than 1 week, they did not keep as long at subsequently higher temperatures as those which had not received the low-temperature treatment. However, this should be expected because even at 35° deterioration takes place, although the rate is very slow. Taking into account the temperature history of each lot of vegetables in its entirety, the product of time and the corresponding temperature coefficients gave results very nearly alike, which is in full agreement with the conclusions derived from the present study. It is safe to say, therefore, that the efficiency of any precooling, handling, and transportation method can be determined with a reasonable degree of accuracy by the method outlined, provided the temperature history of the lot and the temperature constants of the particular vegetable are known.

RATE OF SUGAR LOSSES IN ASPARAGUS, SWEET CORN, AND PEAS AT DIFFERENT TEMPERATURES

In calculating the temperature coefficients given in figure 4, the external appearance of the vegetables was used exclusively as a basis for determining the point at which they reached the stage of complete break-down. It was pointed out, however, that the rate at which visible deterioration takes place is not necessarily the same as the rate at which the edibility of a vegetable changes after harvest. In fact, some preliminary experiments suggested that at least in some vegetables a fairly wide discrepancy exists between these two rates. It was for this reason that a second series of temperature coefficients was calculated for asparagus, sweet corn, and peas. These new coefficients were based on the rate at which sugar disappeared from the stored lots.

The rate at which the percentage of total sugars decreased in three different vegetables when held at 35°, 50°, 65°, and 80° F. is expressed in figure 6. It will be noticed that the rate of sugar depletion is by no means uniform. In some cases, notably in sweet corn (*B*), the rate at which the percentage of sugar decreases becomes much slower as the supply diminishes. Under certain conditions there may be an actual increase in the percentage of sugar present. Obviously the quantity of sugar present at any one time depends on the rate at which sugar is oxidized in the process of respiration and at the same time is replenished by the conversion of starch to sugar. These two processes may have different temperature coefficients and both are controlled by the law of mass action.

The calculation of the temperature coefficients was based on the time interval which causes the sugar content to decrease to 70 percent of its original value at any one temperature. The level of 70 percent was chosen arbitrarily because during this period the rate of sugar

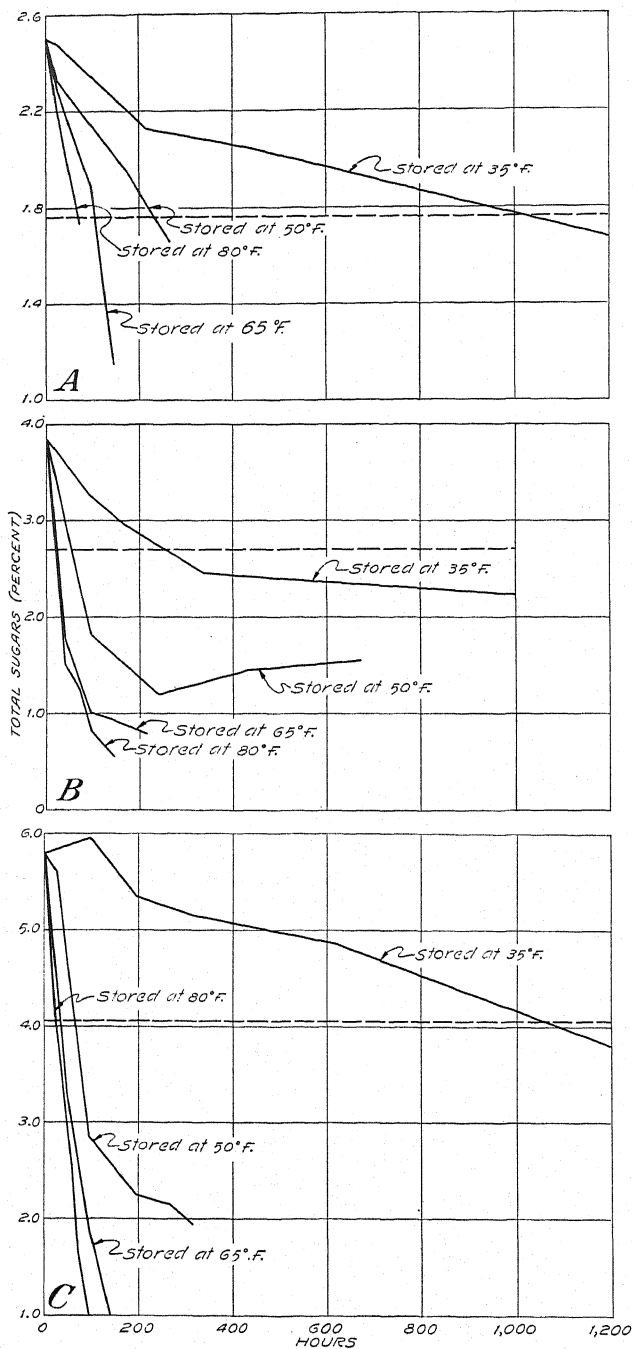


FIGURE 6.—The percentage of total sugars, based on fresh weight, of four lots of asparagus (A), sweet corn (B), and peas (C) when stored at 35°, 50°, 65°, and 80° F. for different periods. In each instance the broken horizontal line indicates the stage at which 30 percent of the original sugar content had disappeared.

loss remains fairly uniform, and also because actual tasting showed that a 30-percent loss of sugar is associated with a very noticeable decrease in sweetness.

Using the method described earlier, the calculations of the temperature coefficients were based on the temperature of the vegetables in storage rather than on the temperature of the storage rooms. The results of these calculations are given in figure 7. As compared with the data given in figure 4 they show that the temperature coefficients for asparagus and peas are more than twice as high when calculated

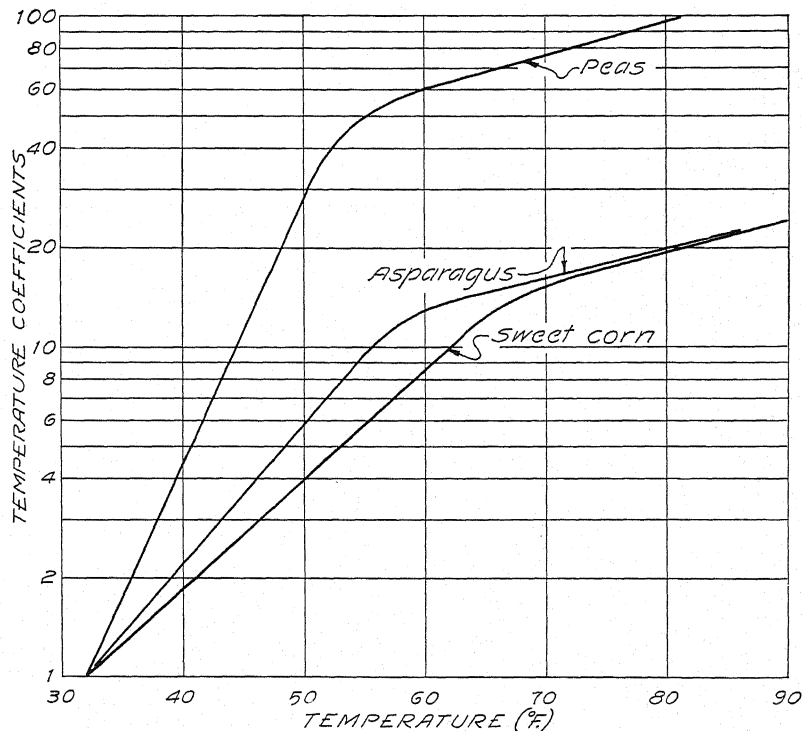


FIGURE 7.—Coefficients indicating the rate at which 30 percent of the original sugar content disappears in asparagus, sweet corn, and peas at different temperatures.

on the basis of sugar depletion as when determined by the rate at which visual deterioration occurs.

The Q_{10} values for the different temperature ranges are given in table 3.

TABLE 3.— Q_{10} values for 3 vegetables based on their rate of sugar depletion at 3 different temperature ranges

Temperature range (° F.)	Peas	Asparagus	Sweet corn
32-50.....	27.5	5.8	3.9
50-68.....	2.6	2.7	3.6
68-86.....	1.5	1.4	1.5

An exceedingly high Q_{10} value was found for peas at the lowest temperature range. The explanation for this is the fact that at temperatures close to the freezing point sugar depletion of peas as a result of respiration is very slow and is almost balanced by the rate at which starch is converted into sugar. Comparing the Q_{10} values at the temperature range of 68°–86° F. in tables 2 and 3, it will be noticed that there is little difference in the rate increment whether deterioration is measured by visual changes or by chemical analysis. Attention should also be called to the fact that the break in the rate curve occurs at about the same temperature in both cases (figs. 4 and 7).

Appleman and Arthur (1) calculated Q_{10} values for sweet corn by a similar method. In general their calculated values are lower than those found in the experiments carried out by the writer. This discrepancy may be accounted for in part by the fact that Appleman and Arthur were dealing with single ears of corn, while the writer

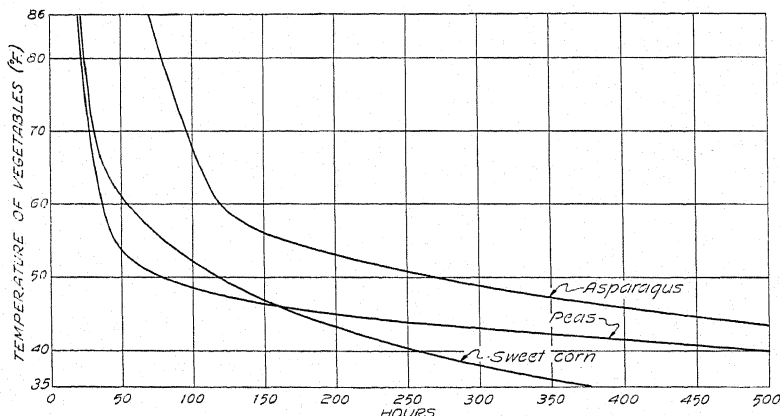


FIGURE 8.—Time-temperature curves showing the time required to produce a 30 percent loss of the original total sugar content of asparagus, sweet corn, and peas when the temperature of the vegetables is that indicated on the ordinate.

obtained his samples from commercial packages. It remains difficult to explain, however, why the Q_{10} values obtained by Appleman and Arthur for the range of 50°–68° F. were consistently lower than they were for the temperature ranges above and below this interval.

In order to show the time required to bring about a 30-percent decrease in the sugar content at any given temperature the data were expressed in the form of time-temperature curves (fig. 8) similar to those presented in figure 5. It is of practical significance that at all temperatures the rate of sugar losses in sweet corn and peas is more than twice as rapid as in asparagus. Furthermore, it should be pointed out that at high temperatures, peas and sweet corn lose their sugar at nearly the same rate, while at low temperatures the sugar losses are retarded much more in peas than in sweet corn. Comparing the time-temperature curves in figures 5 and 8 for the same vegetable, it becomes apparent that at any given temperature the time required to bring about a 30-percent decrease in the sugar content of asparagus is roughly the same as the period necessary for complete deterioration, as measured by external appearance. On the other hand, peas at an

average temperature of 85° F. lose 30 percent of their original sugar content in less than 20 hours, whereas they do not become unmarketable on account of their external appearance until the end of 110 hours. In other vegetables the discrepancy between the rate of sugar depletion and that of visible deterioration may be even more pronounced. Parker and Stuart (8), for instance, have shown that even at room temperature the percentage of sugar in snap beans actually increases slightly throughout the storage period as a result of a rapid rate of hydrolysis of starch to sugar.

SUMMARY

Seven different vegetables were held in storage at 35°, 50°, 65°, and 80° F. Accurate records were taken of the temperature of the vegetables themselves and of the time required to cause complete deterioration of the different lots.

When stored in commercial containers, the temperature of the vegetables may vary widely from the temperature of the room in which they are stored.

Based on the experimental data, a series of temperature coefficients, Q_{10} values, and time-temperature curves were calculated to show the integrated rate at which these vegetables deteriorate at different temperatures and also to show the rate of acceleration of this process when the temperature is raised by a definite number of degrees.

It was found that the temperature coefficients, when plotted against temperature on a semilogarithmic scale, give two fairly straight lines which intersect between 50° and 65° F. The rate of increment in deterioration as a result of rising temperatures was always higher in the lower than in the upper temperature range. Corresponding differences were found in the Q_{10} values, which varied from 2.48 to 4.12 in the temperature range of 32° to 50° with a corresponding variation of 1.58 to 2.03 at temperatures between 68° and 86°.

A comparison of the Q_{10} values in the lowest temperature range showed that in the seven vegetables studied, the Q_{10} values were appreciably lower for those vegetables that usually are considered as being highly perishable than for celery and brussels sprouts which are regarded as less perishable. This suggests that cold storage increases the life of easily perishable vegetables not nearly so much as it does that of the less perishable ones.

The findings emphasize the fact that the Q_{10} values for the rate of deterioration vary not only with different vegetables but apply only to the temperature range for which they were calculated from experimental data.

The time-temperature curves show that the period for which vegetables can be held in storage is lengthened only very slightly when the temperature is lowered from 80° or 90° to 55° F. On the other hand, the life span may be increased very decidedly by a corresponding lowering of the temperature below 55°. Any precooling method, therefore, is of little use unless cooling to temperatures below 55° can be accomplished.

Temperature coefficients and time-temperature curves may be used to predict the length of total storage life of a vegetable. They also furnish a basis for calculating the effectiveness of different pre-

cooling or transportation methods, provided records are available to show the temperature changes occurring in the vegetables themselves.

Holding vegetables at a temperature of 35° F. from a few days to several weeks had no noticeable effect on the subsequent rate of break-down when these vegetables were later transferred to higher temperatures.

The rate of visible break-down and the rate of deterioration in eating quality are not necessarily the same. The Q_{10} values of asparagus, sweet corn, and peas differed widely, depending on whether the rate of deterioration was measured by visible break-down or by the rate at which sugar depletion occurred in these vegetables. A Q_{10} value of 27.5 was calculated for the increment in the rate at which peas lose 30 percent of their original sugar content in the temperature range between 32° and 50° F. On the other hand, the Q_{10} values for this same process were found to be very low in the upper temperature range, varying from 1.4 to 1.5 in the three vegetables studied.

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DIFFERENTIAL SURVIVAL OF ALFALFA STRAINS UNDER AN ICE SHEET¹

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INTRODUCTION

Winter injury of alfalfa (*Medicago sativa* L.) appears principally in three forms in the Great Lakes region, namely, heaving, direct damage from low temperatures, and "smothering" under ice sheets. Data are not available on the relative destructiveness of these three agencies. Heaving is common and may be an important cause of impairment of stands. Willard, Thatcher, and Cutler (10)² attribute most of the winter killing of alfalfa in Ohio to it. Considerable injury from low temperatures in the absence of ice sheets likewise occurs. The most devastating losses, however, in southern Wisconsin at least, result from ice sheets. When these form directly on the soil surface, that is, without an intervening layer of snow, they are apt to be especially destructive. Bacterial wilt, caused by *Phytophthora blight* (L. McC.) Bergey et al., has made serious inroads in parts of this region during the last decade and adds to the complexity of the winter-injury problem. Jones (?) has pointed out that, when sources of infection are about the same, the disease is likely to be much worse in injured than in uninjured fields.

A major part of the change in acres of alfalfa cut for hay in Wisconsin between 1936 and 1937 is attributable to an ice sheet which occurred during the intervening winter. In an area comprising 21 southeastern counties there was a net loss of about 250,000 acres, or 43 percent. The change in this direction reached its maximum in Jefferson and Waukesha Counties, where 80 percent of the alfalfa was destroyed.

The ice sheet formed January 6 to 8, 1937, when 1.54 inches of rain fell at Madison at air and soil temperatures which resulted in its freezing as it reached the surface. The duration of the ice sheet varied somewhat depending on locality and exposure, but it was not until February 13 that level areas were free around Madison. The countryside was encased in heavy ice, therefore, for about 5 weeks. The storm affected all the southern two-thirds of Wisconsin except the western counties near the Mississippi and St. Croix Rivers, where ice was not formed. When it became evident in April that alfalfa had been severely damaged by the ice, plans were made to measure the amount of injury in 42 strains in a replicated planting in the breeding nursery. Although the plot had been laid out with another purpose in view, it proved to be well suited for a study of this problem.

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² Italic numbers in parentheses refer to Literature Cited, p. 71.

THE IRREGULAR DISTRIBUTION OF ICE-SHEET INJURY

Ice-sheet injury tends strongly to occur in patches. This fact is well illustrated by the condition of the experimental plot in question. The approximate relative position of each surviving individual was recorded on quadrillé paper in May 1937. The distribution of the survivors over the area is shown in figure 1. The position of each

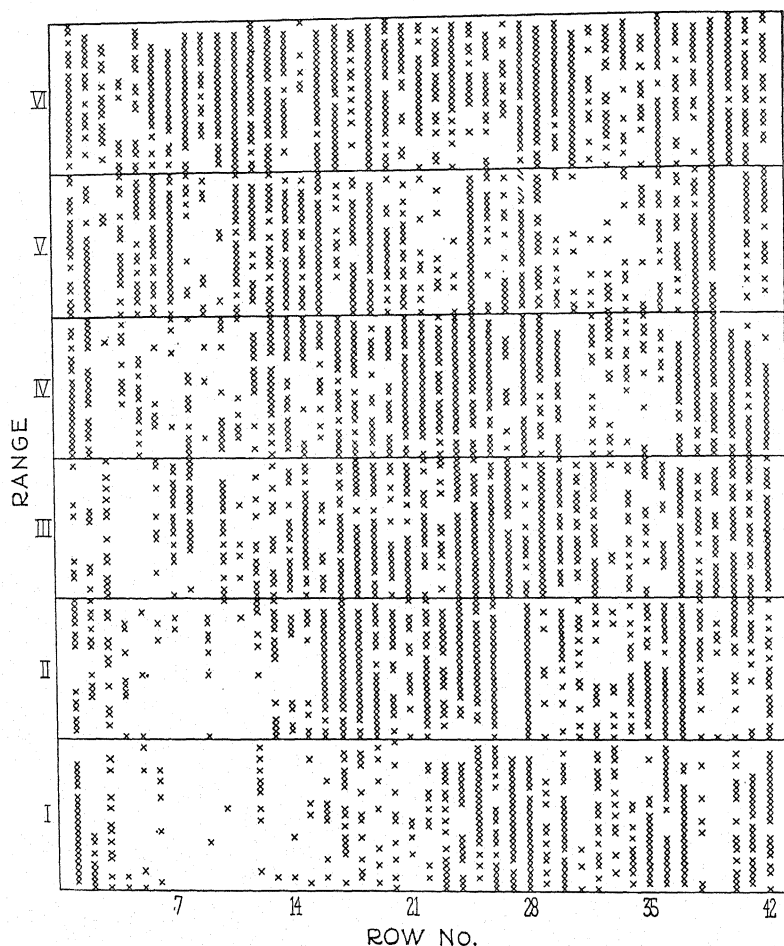


FIGURE 1.—Distribution of surviving alfalfa plants in the spring of 1937 on the experimental plot at Madison, Wis., following ice-sheet injury.

strain in each replication is given in table 1. Although the different strains are scattered on the plot at random, with certain restrictions to be pointed out later, it is readily apparent from the diagram that, regardless of strain, many more plants were killed in some areas than in others nearby. It might be inferred from the patchy character of ice-sheet injury that differences in the hereditary constitution of individual strains or plants are unimportant in determining survival. Indeed, the general appearance of damaged commercial fields, and the fact that, rather commonly, varietal trials afford no positive

evidence of such a relation, might easily lead one to conclude that genetic differences in resistance to ice sheets are entirely wanting. The question, however, is not so easily dismissed. Field observations under farm conditions can rarely be expected to meet the requirements of the case, and it is difficult to obtain competent evidence even from an experimental trial. The chief reason for the latter is the lack of control over the ice-sheet variable.

TABLE 1.—*Planting arrangement of the alfalfa strains replicated in the experimental plot at Madison, Wis.¹*

Range	Alfalfa strain Nos. planted in various rows of—																					
	Column I							Column II							Column III							
VI.....	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
V.....	22	31	26	34	24	40	29	17	36	28	39	30	25	37	23	2	27	41	7	35	5	
IV.....	11	39	27	21	23	38	23	5	35	41	26	19	33	29	42	14	36	10	34	6	13	
III.....	42	14	36	28	18	25	33	6	15	34	27	3	32	40	1	38	30	39	8	12	31	
II.....	13	19	9	30	16	35	10	1	22	18	38	23	20	4	28	33	24	29	40	26	37	
I.....	8	17	37	12	41	20	15	42	24	16	2	31	21	7	11	3	32	25	9	22	4	

Range	Alfalfa strain Nos. planted in various rows of—																					
	Column IV							Column V							Column VI							
VI.....	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	
V.....	42	38	14	8	12	4	13	1	21	9	18	16	10	6	32	19	33	11	15	3	20	
IV.....	9	3	31	37	30	18	40	4	8	15	20	22	7	12	28	1	17	2	24	16	25	
III.....	2	21	16	20	7	19	35	11	24	17	41	23	13	37	5	26	9	22	4	10	29	
II.....	11	34	6	17	41	15	32	42	25	2	39	5	3	36	8	31	14	27	7	21	12	
I.....	1	39	33	29	10	36	5	27	40	38	19	28	14	26	6	30	23	18	34	13	35	

¹ The strains were replicated 6 times in rows 12.5 feet long and 24 inches apart, the plants being spaced at approximately 6 inches in the row. Each strain occurred once in each of the 6 ranges (running north and south) and once in each of the columns. Each column was 7 rows wide.

Ice-sheet injury apparently is a complex phenomenon. There are formidable obstacles in the way of a planned test for genetic differences between strains. We are virtually dependent at present upon such evidence as is afforded by the occasional field trial which happens to encounter the conditions under which genetic differences, if present, may appear. What these conditions are cannot be fully specified. Two requirements, however, may be stated: (1) The average level of injury must be such that a minimum number of strains fall in the extreme classes of 100-percent killing or 100-percent survival; (2) in order that an informative analysis of the results be made, the experiment must be so designed as to permit the segregation of possible hereditary differences from other, frequently large, sources of variability. As the level of injury in the field is beyond control, and the time, place of occurrence, and duration of ice sheets cannot be readily predicted, there is a considerable element of chance in obtaining significant data on the problem. That such evidence is available in the present study is due to the circumstance that the main requirements, as laid down above, happen to have been met.

Assuming that alfalfa strains vary in resistance to ice sheets, over how wide a range of test conditions is a difference maintained? General observations indicate that differences in survival between genotypes disappear as the severity of an ice sheet reaches a certain level. In commercial fields, for example, sown to one of the ordinary varie-

ties, which comprises a diverse assemblage of genotypes, there may be a sharp line of demarcation between areas in which, on the one hand, all the plants are killed and, on the other, most of them live. In other words, the magnitudes of the differences in survival between strains are a function of the severity of the conditions, and above a certain point, hereditary variations, which may be significant at lower levels, are completely overridden. This relationship must be considered one of the most important aspects of the problem.

The present experimental data are based on a single trial in which the plants were exposed rather uniformly to a particular set of ice-sheet conditions. Were these conditions severe, moderate, or mild? If the relation postulated in the preceding paragraph holds, it is necessary to answer this question in order that the findings be understood in their bearing on the general problem of ice-sheet injury.

THE RELATIVE SEVERITY OF THE ICE ON THE EXPERIMENTAL PLOT

The damage done to alfalfa by the 1937 ice sheet varied greatly over the extensive area within which the experimental plot lay. It is possible to establish with fair accuracy the amount of damage done by the ice at Madison where the experimental plot was located, as compared with that in the surrounding sections, from the assessor's reports³ of acres of alfalfa cut for hay in 1936 and 1937. The net change during this interval is shown for Wisconsin by counties in figure 2. Each black dot represents a net loss of 200 acres and each hollow circle a net gain of the same amount. Furthermore, from the replies to a questionnaire sent by the Federal-State Crop Reporting Service for Wisconsin to local reporters in February 1937 concerning ice-sheet conditions on their respective farms, the position of the plot is revealed relative to an area in which the destructive effects of the ice were more or less offset by an important protective factor, namely, a sub-layer of snow.

South of a straight line drawn in figure 2 from the southeastern corner of Door County to the southwestern corner of the State the soil tended to be free from snow at the time the ice sheet developed; north of this line a more or less continuous covering of snow was present on top of which the ice formed as a crust. More accurately speaking, the line passes through a transition zone between an area uniformly covered with snow to the north and a snow-free area to the south.

One is immediately impressed with the relationship between the line dividing the region in which losses occurred from that in which gains are shown and the boundary of the snow cover at the time the ice sheet was formed. The two lines coincide, so far as the available records permit of their positions being determined. The ice was extremely destructive where it formed directly on the soil and much less injurious where there was snow underneath.

The experimental plot at Madison was located in the transition zone between the area bare of snow and that covered with snow when the ice formed. This is clearly brought out in figure 3 in which the net changes in acreage harvested for hay in 1936 and 1937 for Dane

³ The writers are greatly indebted to Dr. W. H. Ebling, senior agricultural statistician, Federal-State Crop Reporting Service for Wisconsin, for supplying these data in advance of publication. He has likewise made available for the writers' use the replies to the questionnaire on ice-sheet injury.

County are plotted by townships. The mortality on the plot was high, as will be seen later, but the level of injury was below the point which led to significant acreage decline elsewhere in the State. An appreciation of this fact is important in interpreting the data obtained.

ORIGIN OF THE STRAINS

The 42 strains whose reaction to the ice sheet was studied formed a relatively homogeneous group. Two checks were included, one of which was Grimm, a well-known hardy variety widely grown in the

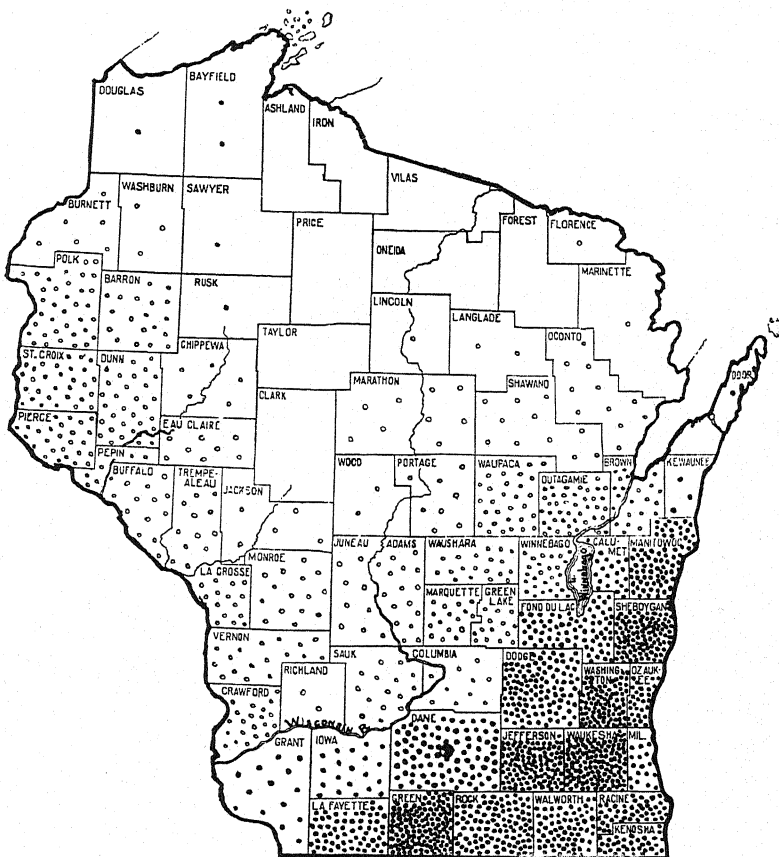


FIGURE 2.—Net change in acres of alfalfa cut for hay between 1936 and 1937 in Wisconsin by counties. Each dot represents a net loss of 200 acres over 1936, and each hollow circle a net gain of equal amount. (Based on assessor's reports to Dr. W. H. Ebling, senior agricultural statistician, Federal-State Crop Reporting Service for Wisconsin.)

State, and the other, Hardigan, which is similar in type and cold resistance. The remaining strains, for the most part, were either single plant lines (once, twice, or three times self-pollinated) selected from the Grimm variety on the basis of cold resistance, as measured by an artificial freezing test, or single, three-way and double hybrids involving these strains. One strain similarly selected from the Cossack variety was also represented, both as a single plant line and in a few

of the hybrids. A five-times self-pollinated line derived from Turkestan alfalfa was the only stock included which was resistant to bacterial-wilt. In a rather drastic test involving artificial inoculation with the bacterium this strain had previously been found to contain approximately 50 percent of highly wilt-resistant individuals. All the seed used in the plantings, except that of the check varieties, was produced in the greenhouse by controlled hand-pollination.

The relative uniformity of origin of the strains under test is important in gaging the significance of the results. Obviously, it means

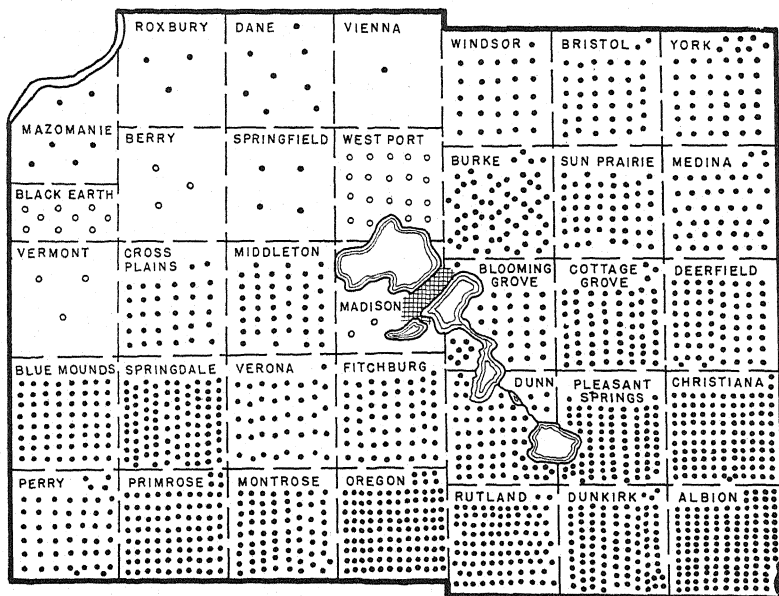


FIGURE 3.—Net change in acres of alfalfa cut for hay between 1936 and 1937 in Dane County, Wis., by townships. Each dot represents a net loss of 10 acres over 1936, and each hollow circle a net gain of equal amount. (Based on assessor's reports to Dr. W. H. Ebling, senior agricultural statistician, Federal-State Crop Reporting Service for Wisconsin.)

much more to the breeder if differences in resistance to ice-sheet injury exist in strains derived from varieties already rather well adapted to the region than if such differences can only be demonstrated in more widely diverse stocks.

THE EXPERIMENTAL PLOT

The plot was laid out in the form of a modified Latin square. The strains were replicated six times in rows 12.5 feet long and 24 inches apart, the plants being spaced at approximately 6 inches in the row. Each strain occurred once in each of six ranges, running north and south, and once in each column, seven rows wide, running at right angles. Except for these restrictions, the strains were grown in random order as shown in table 1.⁴

⁴ This type of arrangement was suggested by Student (9) and is frequently referred to as the "semi-Latin square." Although this arrangement provides efficient, unbiased estimates of the true percentage mortalities for the 42 strains, Yates (11) has pointed out that designs of this type, when analyzed according to the usual analysis of variance procedure appropriate to a Latin square, suffer from a biased error mean square, and Gouliden (6) has evaluated an expression for this bias. The probable nature of the bias in the present experiment and its effect on the inferences drawn are discussed later (p. 67).

An even slope, nearly parallel to one diagonal, gave the plot a relatively uniform relief. The fall along this diagonal was 2.3 feet in approximately 115 feet. Excellent surface drainage was thus provided. Only in one corner, where a slight depression crossed the plot, did moisture conditions appear to change significantly. Mortality in this area was especially high.

The soil was fertile Miami silt loam, in good tilth. It had been limed a few years earlier to correct a slight acidity. The natural underdrainage was good.

The seed was sown by hand in early summer, 1935, and, with a small amount of replanting, an excellent stand was obtained. One cutting, bearing a crop of seed, was taken in July 1936. The summer was extremely hot and dry, and water being available, the plot was irrigated once after the seed pods had formed. Fall rains were abundant, however, and the plants entered the winter of 1936-37 bearing a large growth of shoots.

BACTERIAL WILT ON THE EXPERIMENTAL PLOT

The soil in this area is known to be infested with *Phytophthora insidiosa*, the causal organism of bacterial wilt of alfalfa. The extent to which the disease contributed to the killing of plants which became apparent after the ice sheet in early 1937 is somewhat uncertain. Two plants were observed in the late summer of 1936 which showed unmistakable symptoms of bacterial wilt. While no plants were dug, a survey of the plot at that time failed to reveal any more diseased individuals. Considering that the planting was only in its second year of growth, it is unlikely that the disease had progressed very far even though many individuals may have become slightly infected before the winter of 1936-37. Because the one wilt-resistant strain included in the test, No. 15, was most severely damaged (96-percent mortality) while wilt-susceptible strains were damaged as little as 12 percent, it appears to be a safe assumption that wilt was not a major cause of death on the experimental plot.

MORTALITY ON THE EXPERIMENTAL PLOT

Counts were made May 8 and 9, 1937, of the living and the dead plants on the experimental plot. The position of the dead individuals was clearly marked by stem residues, as growth during the previous fall had been abundant. Classification was made without difficulty for the most part. Between the plants which had been killed outright and those which were clearly in a condition to survive, however, there was a small group less easy to place. These plants bore one or more living, but weak, shoots and obviously had suffered severe injury. If it appeared that such a plant was more likely to die than to survive, it was entered in the "dead" class; otherwise it was counted as living.

The total number of plants and the percentage killed have been collected according to strain and range in table 2. Considerable variation is noted between the mean values. Strain 3 is the Grimm check, in which 42 percent of the plants were killed, on the average. Hardigan, the other check variety, is strain 9 and showed 47.5 percent mortality. The remaining strains range from 12 percent to 96 percent killing, the general mean for all strains being 42 percent.

TABLE 2.—Mortality in alfalfa strains on experimental plot at Madison, Wis.

Strain no.	Total plants and percentage killed in range—														Total plants	Mean percentage of dead plants
	I		II		III		IV		V		VI					
	Total plants	Killed	Total plants	Killed	Total plants	Killed	Total plants	Killed	Total plants	Killed	Total plants	Killed				
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent		
1	25	60	26	100	25	20	23	17	25	28	26	15	150	40.00		
2	24	100	24	50	25	44	25	8	24	4	26	38	148	40.67		
3	25	72	25	40	21	52	24	17	24	29	24	42	143	42.00		
4	25	44	21	67	24	17	24	17	25	48	15	40	134	38.83		
5	25	4	25	60	24	63	25	56	24	38	26	38	149	43.17		
6	25	16	25	16	23	39	24	25	25	64	23	13	145	28.83		
7	25	88	25	40	25	12	23	61	25	8	22	14	145	37.17		
8	25	20	25	12	25	16	23	17	24	4	25	8	147	12.83		
9	25	76	24	58	24	33	24	4	25	72	24	42	146	47.50		
10	26	31	21	90	25	28	24	21	25	68	26	12	147	41.67		
11	22	82	26	15	25	0	24	17	25	8	25	4	147	21.00		
12	25	92	25	32	25	52	25	72	25	48	25	8	150	50.67		
13	22	14	25	40	26	46	25	8	25	12	25	4	148	20.67		
14	25	60	25	36	25	76	24	46	25	80	25	28	149	54.33		
15	25	100	24	96	25	100	24	100	23	100	25	80	146	96.00		
16	26	96	23	87	25	12	26	27	25	68	24	4	149	49.00		
17	27	70	25	20	24	33	25	8	25	64	26	4	152	33.17		
18	23	91	23	96	16	100	24	54	24	88	23	52	133	80.17		
19	26	31	25	60	25	8	23	48	25	36	26	8	150	31.83		
20	24	71	25	44	24	8	25	48	25	36	25	12	148	36.50		
21	26	96	25	52	26	54	25	68	25	64	25	40	152	62.33		
22	25	56	23	70	24	25	25	40	25	24	25	24	147	39.83		
23	25	76	26	69	24	88	25	84	25	28	26	35	151	63.33		
24	23	96	25	4	24	21	25	12	23	30	26	19	146	30.33		
25	26	62	25	36	26	73	25	32	25	28	26	42	153	45.50		
26	23	30	23	39	23	0	24	75	25	92	24	33	142	44.83		
27	26	58	23	78	25	84	25	92	24	63	26	54	149	71.50		
28	25	52	25	68	21	100	24	75	24	83	25	8	144	64.33		
29	25	16	25	8	25	12	25	24	25	8	25	4	150	12.00		
30	25	16	25	68	26	19	25	8	24	20	26	23	151	27.17		
31	27	63	24	0	24	21	25	4	24	25	24	0	148	18.83		
32	27	44	25	4	25	56	25	48	25	32	26	58	153	40.33		
33	24	29	24	13	23	35	25	20	25	12	25	40	146	24.83		
34	25	32	23	30	20	30	22	36	24	29	25	44	139	33.50		
35	26	31	25	92	25	48	24	88	24	33	23	48	147	56.67		
36	25	4	25	24	25	48	25	16	23	70	26	23	149	30.83		
37	23	48	25	44	24	46	25	12	24	17	25	40	146	34.50		
38	21	86	21	95	22	73	22	86	24	71	24	42	134	75.50		
39	26	31	24	63	25	16	25	24	24	21	26	4	150	26.50		
40	26	19	24	21	25	40	24	8	24	8	25	8	148	17.33		
41	26	81	24	4	25	20	26	58	24	21	23	9	148	32.17		
42	25	100	25	76	25	76	25	52	24	58	26	38	150	66.67		

The first question to be considered is whether the strains differ significantly in survival. Proceeding according to the analysis of variance technique customary with a Latin-square arrangement, the variation in mortality about the general mean, as measured by sum of squared deviations, has been broken down into four components with their associated degrees of freedom as shown in table 3.⁵ If the

⁵ The variance of an observed proportion P' is known to be $P(1-P)/N$, where P is the theoretical proportion which it samples and N is the number of observations upon which it is based. Consequently if the data in an experiment are expressed in percentages, and if the percentages differ widely among themselves—suggesting differences in the values of P sampled—then they may be of widely differing accuracy as measured by their variances. One of the assumptions underlying the analysis of variance technique is that the observations of the various classes are distributed about their true mean values, equal or different, with the same variance. Clearly this fundamental assumption is violated when percentages are used if differing mean values are admissible, owing to column and range effects. The extent to which error has been introduced can be determined by transforming the percentages to some new variable which will have a constant or nearly constant variance over the entire set of data at hand. It can be shown mathematically that the appropriate transformation is to the new variable θ defined by $\theta = \arcsin \sqrt{P}$, and this has been used by Bartlett (1, 2), Bliss (3, 4), and Cochran (5), in the papers cited below. The present analysis was repeated in these new units. It will be sufficient to indicate here the F values obtained in this analysis—they have the same respective significance levels. They are 12.18, 19.61, and 6.52 for ranges, columns, and strains, respectively. Clearly one can draw the same inferences from these as from the original values; it is quite remarkable that the changes have been so slight.

variation over the field is such as would arise through random sampling from a population in which column, range, and strain effects are nonexistent—the null hypothesis being postulated—and if the Latin-square type of analysis were exactly applicable, then the mean squares in column 4 in table 3 would have the same expectations. Actually the residual mean square, 532.94, is biased to some extent, as was mentioned in the footnote on page 64, the exact extent of the bias being unknown. From Goulden's assessment, however, it appears that this type of bias is generally positive so that the value 332.94 is probably too large. Consequently, if the mean square between strains is significantly larger than 332.94, it is probably significantly larger than the true, but unknown, estimate of residual variance. Similarly for the other comparisons.

TABLE 3.—*Analysis of variance for percentage of alfalfa plants killed on experimental plot at Madison, Wis., during the winter of 1936-37*

Variance due to—	Degrees of freedom	Sum of squares	Mean square	F	1 percent ¹ values
Ranges.....	5	20,391	407.20	12.25	3.11
Columns.....	5	32,350	6470.00	19.43	3.11
Strains.....	41	86,093	2099.83	6.31	1.28
Residual.....	200	66,587	332.94	-----	-----
Total.....	251	205,421	-----	-----	-----

¹ Snedecor (8, pp. 174-177).

As was shown in the column labeled "*F*" (table 3), the mean square between strains is over six times the residual mean square. Comparing this with the 1 percent value of *F*, 1.28, given in the last column of table 3, the existence of strain effects is strongly suggested, since the mean square for "between strains" would exceed the residual mean square about once in 100 trials were the null hypothesis true. In brief, the observed event is highly improbable on assumption of the null hypothesis; therefore if the latter be rejected on this evidence, there is only 1 chance in 100 that it is rejected falsely. In this sense the analysis evidences the existence of differences between strains.

In like manner the analysis indicates the existence of differences between columns, and between ranges. It should be noted in this connection that when the significance of one effect is demonstrated this does not invalidate the test for another. This is an important property of the analysis of variance technique.

Since the Latin-square type of analysis is not strictly applicable to the lay-out at hand, it is of interest to inquire how the variability arising from strain differences and residual error, as measured by the between strain mean squares, compares with that arising from all sources other than between strain differences. To do this the sums of squares for ranges and columns are combined with that labeled "residual" in table 3, and the total divided by 210 degrees of freedom, yielding a mean square of 568.70. Comparing the between strains mean square with this, *F* is found to be 3.69, a value significant at the 1-percent level of significance. In other words, the evidence strongly supports the conclusion that there are significant differences in mortality between strains.

One may next inquire which comparisons are significantly different. Accepting the respective means as the best estimates of mortality for the strains concerned, it is desired to determine what difference between means is needed in order that it may be adjudged significant at a stated level of significance. This difference is calculated as follows:

$$(\bar{x}' - \bar{x}'')_{\text{percent}} = t_{\text{percent}} \times S \sqrt{\frac{1}{N_1} + \frac{1}{N_2}}$$

in which $(\bar{x}' - \bar{x}'')_{\text{percent}}$ is the magnitude of a difference necessary for significance at the level of significance (e. g., 5 percent, 1 percent) chosen, t_{percent} being the tabulated value for 200 degrees of freedom and the particular level of significance (i. e., percent desired, S the square root of the residual mean square in table 3, and $N_1 = N_2 = 6$. In this manner the 5 percent and 1 percent minimal significant differences were obtained and were found to be

$$(\bar{x}' - \bar{x}'')_{0.05} = 20.74 \text{ percent and } (\bar{x}' - \bar{x}'')_{0.01} = 27.25 \text{ percent}$$

The mean percentage of plants killed in Grimm (strain 3), a standard variety for the section, is 42.0, as shown in table 2. The others may be conveniently compared with it. The upper and lower bounds for significant difference from Grimm are as follows: For a 5-percent level of significance, upper bound 62.7, lower bound 21.3; and for a 1-percent level of significance, 69.2 and 14.7, respectively. This means that any strain in which the mean percentage of killing is either at least 62.7, or not more than 21.3, may be considered to be significantly different from Grimm at the 5-percent level of significance. Similar deductions may be made for the 1-percent values.

The positions of the 41 other strains in relation to that of the Grimm variety are depicted in figure 4, in which the limits of significance, according to the above criteria, are indicated. The differences in the amount of killing shown between most of the strains and Grimm are no larger than might frequently be expected to result from random sampling alone. There are several strains, however, some of which are highly susceptible to ice-sheet injury, others relatively resistant, which appear to differ significantly in this respect from the Grimm variety. The occurrence of strains which appear to be able to withstand ice better than this standard commercial type is of particular interest. There is little room for doubt that nos. 8 and 29 which showed 12.8 percent and 12.0 percent killing, respectively, are in the latter group; strains 11, 13, 31, and 40, showing 21.0, 20.7, 18.8 and 17.3 percent, respectively, are very probably so.

SURVIVAL OF INBRED AND HYBRID STRAINS

The average amount of killing in seven inbreds, based on six observations each, is 44.4 percent. The average percentage of killing in the 17 hybrids into which these inbreds enter, based on six observations each, is 37.4. The difference is probably significant, the calculated value of t^6 being 2.10 whereas the tabulated value for 200 degrees of freedom is 1.97 for the 5-percent level of significance and 2.60 for the 1-percent level. It is a reasonably safe conclusion, there-

⁶ t is computed from a simple rearrangement of the formula on this page, but in the present instance $N_2 = 42$, $N_1 = 102$, and $(\bar{x}' - \bar{x}'')$ is the observed difference between the two means.

fore, from the data at hand, that hybrid strains in general survive better than inbred strains, the magnitude of the difference being approximately 7.0 percent.

DISCUSSION

The data which have been presented show that, under the conditions of the experiment, hereditary differences between strains were important in determining survival under an ice sheet. This finding is the more significant because the stocks under test were relatively

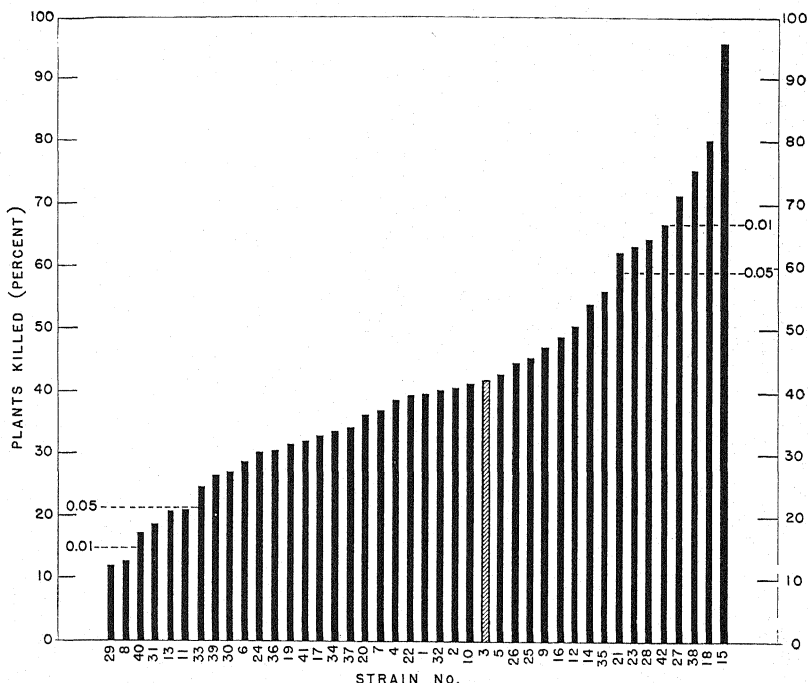


FIGURE 4.—The 42 strains of alfalfa on the experimental plot at Madison, Wis., arranged in ascending order of percentage of plants killed by the ice sheet. The Grimm check variety is represented by the crosshatched bar. The upper and lower bounds demarking percentages of killing significantly different, statistically, from the value for Grimm are shown for the 0.05 and 0.01 levels.

homogeneous and, with one exception, were derived from varieties considered suitable for the region. The existence in alfalfa of heritable differences of this kind is an interesting and significant fact in itself, but interpretation of the results in terms which have meaning for the plant breeder requires that another factor be taken into consideration, namely, the level of the test conditions. The latter statement is predicated on the hypothesis that as the ice-sheet factor becomes more severe genetic differences in ability to survive are overshadowed and may even disappear. Observation of ice-sheet injury in commercial fields supports this view, and indicates also that such levels of injury are frequently reached in the Great Lakes region.

It has been possible to show that the level of ice-sheet injury in the vicinity of the experimental plot was rather low relative to that in

nearby parts of the State where very severe losses of commercial acreage were experienced. This does not mean that on the test plot much damage was not done. On the contrary, nearly one-half the plants of the Grimm and Hardigan check varieties were killed and the remainder more or less severely injured. But the fact is established that ice-sheet conditions occur which impose a much more rigid test on the alfalfa plant's ability to survive than the one obtaining in the present experiment. The conclusion at which we arrive, therefore, must be a restricted one, namely, that there are observable significant differences between strains in ability to withstand a moderately severe ice sheet. What happens above this level remains for future experiments to determine.

If genetic differences in capacity to survive are overridden under the more destructive ice sheets, it is worth while to point out the biological implications of the fact. Adaptation, among other things, implies the possession of heredity which enables the organism to withstand the adverse elements in its environment. The extent to which the action of an unfavorable external agent is unopposed by the genotype is a measure of the organism's lack of adaptation in that particular respect. If, therefore, the effect on alfalfa of the more severe ice sheets is determined largely by external conditions, heredity playing a minor role, it merely means that the plant is correspondingly unadapted in this regard.

SUMMARY

Ice sheets, which form on the ground when rain falls at freezing temperatures, frequently cause great damage to alfalfa in Wisconsin and adjacent states. Very extensive damage resulted from an ice sheet during January and February of 1937 which, however, provided an unusual opportunity to study the reaction of alfalfa to this type of injury in an experimental plot containing 42 strains, located at Madison, Wis. Madison was in a transition zone between the severely damaged area to the south and east where the ground was bare, and the moderately damaged area to the north and west where the ground was covered by a protective layer of snow when the ice formed.

Many of the 42 strains were either self-fertilized selections from Grimm, or their hybrids. The parental lines had been selected on the basis of their cold resistance as measured by an artificial freezing test. Both the Grimm and Hardigan varieties were included as checks. The average mortality on the experimental plot was 42 percent, but values for individual strains ranged from 12 to 96 percent. A statistical study of the data indicates that significant differences in survival between strains occurred and, furthermore, that several strains were more resistant than either Grimm or Hardigan. Strains of hybrid origin were more resistant than their inbred parents.

The reaction of alfalfa strains to an ice sheet is dependent upon the severity of the ice-sheet complex. In the present instance the conditions imposed upon the experimental plot were mild as compared with those applying to a large part of southeastern Wisconsin. Under the more severe conditions significant differences established in the present study would no doubt be obliterated.

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TRANSMISSION OF SUGARCANE MOSAIC BY APHIDS¹

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INTRODUCTION

The spread of sugarcane mosaic in the field frequently is difficult to explain on the basis of transmission by the corn leaf aphid (*Aphis maidis* Fitch) alone. It seems likely that other insects play a part in the spread of the disease. Since Brandes (2)² presented evidence showing that *A. maidis* could act as a vector of sugarcane mosaic, the problem of insects in relation to the transmission of the disease has been studied by a number of investigators. Kunkel (6, 7) published the results of studies in Hawaii which further showed the ability of *A. maidis* to transmit sugarcane mosaic. Chardon and Veve (3) conducted experiments in Puerto Rico which convincingly demonstrated that *A. maidis* could transmit sugarcane mosaic under field conditions. They also reported an experiment involving six plants in which four transmissions were obtained with a then unidentified species of the aphid genus *Carolinaia*. This experiment was not accompanied by controls, and it is possible that the aphid involved was *Carolinaia cyperi* Ainslie. Ingram and Summers (5), in work conducted in Louisiana during the years 1933-35, found that sugarcane mosaic could be transmitted from diseased to healthy plants by the rusty plum aphid (*Hysteroneura setariae* (Thomas)).

A number of different species of aphids were used by the writers in transmission experiments. Two other than *Aphis maidis* definitely were found to act as vectors of sugarcane mosaic and some evidence was obtained to indicate that still other species might be involved. The results of these studies are presented herein.

TRANSMISSION EXPERIMENTS

INSECTS USED

With the exception of a small number of miscellaneous tests, which are discussed in a subsequent section of this paper, the transmission experiments were confined to four species of aphids—*Carolinaia cyperi*, *Hysteroneura setariae*, *Sipha flava* (Forbes) (the yellow cane aphid), and *Aphis maidis*—the last-named species being used primarily to serve as a control in experiments involving other species.

A stock supply of aphids was obtained by placing large numbers of individuals, collected in the field, on plants growing in cages and then, after vigorous colonies had become established, transferring a single female to a fresh plant to start a colony from which individuals could be taken for use. By this procedure a homogeneous stock of aphids was obtained and used in all the experiments.

¹ Received for publication February 11, 1939. These studies were conducted in cooperation with the Puerto Rico Experiment Station, U. S. Department of Agriculture, at Mayaguez, P. R., where various facilities, including office and laboratory space and field plots, were furnished.

² Italic numbers in parentheses refer to Literature Cited, p. 78.

OTHER MATERIALS

The original stock of virus used in these experiments was obtained by selecting a single plant showing well-marked mosaic symptoms from a group of plants grown from seed pieces from diseased plants. All subsequent inoculations were made either from this plant or from other plants infected in serial transfers from the same source. All diseased plants were grown in porous clay pots.

The experimental plants were grown both from cuttings and from seed, in either porous clay pots or wooden flats having a soil depth of from 6 to 8 inches. In the case of cuttings some of each of the two sugarcane (*Saccharum officinarum*) varieties SC 12 (4) and BH 10 (12) were used, but the seedlings were grown exclusively from seed from the variety SC 12 (4). The temperature of the growing house averaged about 85° F.

Small tube cages, ranging from 2 to 3½ inches in diameter and from 6 to 14 inches in height and made of celluloid, were used for confining the insects on healthy sugarcane plants. The cage was placed over the entire plant. Aphids were then placed on the plant, and the upper end of the cage was covered with cheesecloth held in position by a rubber band.

METHOD OF HANDLING INSECTS

Transfer of the insects from one plant to another was accomplished by means of either a camel's-hair brush or an aspirator similar to the type described by Kunkel (6) for handling leafhoppers. In each test large numbers of aphids were confined on a diseased plant and then, at the time of making transfers to healthy plants, only those individuals that were observed to be feeding were selected. During the early part of the work from 15 to 20 aphids were confined on an experimental plant, but the number later was increased to 50. The aphids were confined on a diseased plant from 24 to 48 hours and then transferred to a healthy test plant for a similar period. At the end of that time the cages were removed and the plants carefully sprayed with a strong solution of pyrethrum and soap, after which they were transferred to the growing house.

The walls of the growing house, as well as all outdoor cages, were covered with 30-mesh copper screen, and the roof was covered with Celloglass. As a protection against ants or other insects the growing house and outdoor cages were surrounded by moats which were kept filled with either water or oil.

After exposure to infective aphids the experimental plants were examined daily for 30 to 40 days for evidence of mosaic. Plants showing no symptoms up to this time were observed at less frequent intervals for an additional 30 days before being discarded. At all times the number of control plants was equal to the number of exposed plants. Throughout the course of the tests all plants in the growing house were carefully sprayed with a strong solution of pyrethrum and soap at 3- or 4-day intervals. Sugarcane plants suffered no apparent injury as a result of the pyrethrum-soap spray, but nicotine sulphate, which was originally used, caused severe burning.

EXPERIMENT WITH CAROLINAIA CYPERI

Colonies of *Carolinaia cyperi* needed for experimental purposes were reared on the sedge *Cyperus rotundus* in screened cages. When confined on sugarcane plants, particularly on young seedlings, the aphids fed readily during the first 12 to 18 hours, after which they became restless and crawled about over the plants and the sides of the cages. Large numbers, however, survived for 3 or 4 days and some were still alive at the end of 5 or 6 days.

In 30 tests a total of 192 healthy plants were exposed to *Carolinaia cyperi* taken from diseased plants, and 60 of these, or 31.3 percent, developed mosaic. Seventy-three of the plants were grown from cuttings, and 11, or 15 percent, of these became diseased. Of the remaining 119 plants, all of which were grown from seed, 49, or 41.2 percent, became diseased.

The incubation period in plants grown from cuttings ranged from 20 to 42 days, with an average of 27.6 days. In seedlings the range was from 7 to 28 days, with an average of 18.2 days.

EXPERIMENT WITH HYSTERONEURA SETARIAE

In Puerto Rico one of the preferred hosts of *Hysteroneura setariae* is goosegrass (*Eleusine indica*), and since this grass grows well in cages, it was selected as the host plant for rearing the stock supply of this aphid.

One hundred and thirty-seven plants were exposed in 26 tests to infective *Hysteroneura setariae*, and 11, or 8 percent, developed mosaic. Forty-five of the plants were grown from cuttings, and 1 became diseased; 92 were grown from seed, and 10, or 10.9 percent, became diseased.

It will be noted that the percentage of successful transfers with *Hysteroneura setariae* is comparatively low, but the results are considered sufficiently reliable to justify the conclusion that this aphid can act as a vector of sugarcane mosaic. It is evident, however, that it was considerably less efficient as a vector of sugarcane mosaic than either *Carolinaia cyperi* or, as will be shown later, *Aphis maidis*. The results with the latter are in agreement with those obtained by Ingram and Summers (5).

EXPERIMENT WITH SIPHA FLAVA

The technique employed with *Sipha flava* differed from that used with the other species of aphids in that the stock supply was maintained continuously on mosaic-infected sugarcane by means of successive transfers to diseased plants. From this colony aphids were transferred directly to healthy plants. In 9 tests 75 healthy plants, 35 from cuttings and 40 from seedlings, were exposed to *S. flava* taken from diseased plants. None of these developed mosaic symptoms during the 2½ months that they were kept under observation. When 20 to 50 individuals of *S. flava* were confined on a young seedling for 2 days or more, the older leaves usually developed a reddish-brown color, the characteristic injury associated with this species of aphid, and gradually died. Somewhat similar but considerably less severe symptoms resulted when *S. flava* was confined on young plants grown from cuttings.

EXPERIMENT WITH APHIS MAIDIS

Several plants were tried in seeking a host for maintaining a constant supply of *Aphis maidis* in small cages, and dwarf popcorn (*Zea mays* var. *evarta*) was found to be the most satisfactory.

Infective individuals of *Aphis maidis* were confined on 200 healthy sugarcane plants and 69 of the plants, or an average of 34.5 percent, developed mosaic. Of this number 57 were grown from cuttings, and 19, or an average of 33.3 percent, became diseased. The remaining 143 exposures were made on seedlings and 50, or 35.0 percent, of these were infected by transfer of the virus.

EXPERIMENTS WITH OTHER APHIDS

In February and March 1936 some miscellaneous experiments were conducted with three species of aphids that were available on plants growing near the insectary at Mayaguez, all of which were grown from seed. Nine plants were exposed to *Aphis rumicis* L., nine to *Macrosiphum rudbeckiae* (Fitch), and four to *A. nerii* Fonsc. The only plant to become diseased was one in the series exposed to *A. nerii*. Although it is realized that these tests were by no means extensive enough to give conclusive evidence, the implications are of considerable interest.

OCCURRENCE OF THE EXPERIMENTAL APHIDS IN PUERTO RICO

According to available records, *Carolinaia cyperi* is limited in its distribution to tropical and subtropical climates and, so far as known, its host-plant range is confined to certain sedges of the genus *Cyperus*. *C. cyperi* was originally described by Ainslie (1) from specimens collected at Lakeland, Fla., on *Cyperus esculentus*, the common nutgrass, or chufa, of the Southern States. The writers found *C. cyperi* to be quite generally distributed in Puerto Rico, where it appears to colonize only on "coqui," (*Cyperus rotundus*). Coqui constitutes one of the more serious weed pests in Puerto Rico and seems to thrive during favorable seasons on many types of soil. Records taken in the vicinity of Mayaguez, which covered the period from September 1935 to July 1936, showed that this aphid attained its greatest abundance between the extremely wet and the extremely dry season. Coqui grows abundantly in and around sugarcane fields, and during the process of cultivation and otherwise caring for sugarcane plantings it is destroyed, thus forcing the aphids to migrate in search of new host plants.

During certain seasons of the year *Hysteroneura setariae* was found in considerable abundance on a number of grasses growing in the vicinity of cane fields. In some localities in Puerto Rico, particularly along the central southern coast, *H. setariae* has been found to colonize on cane, and fairly heavy local infestations occasionally were observed. The colonies were practically always found at the junction of the leaf blade and sheath.

Sipha flava is present in Puerto Rico at all seasons of the year, but according to records it is most abundant during the dry season and in the more arid regions. In the section of Puerto Rico having comparatively little rainfall, which, generally speaking, includes the south side of the island, *S. flava* is a pest of considerable importance through-

out the year, but the greatest damage occurs on young sugarcane during the spring months.

Aphis maidis is not known to feed on sugarcane to any appreciable extent in Puerto Rico. In rare instances small colonies have been observed to survive for a few weeks on small sugarcane plants in cages. In the transmission studies it was found that a few *A. maidis* individuals would survive on sugarcane plants for a week or 10 days, but the majority died within 4 to 6 days.

DISCUSSION OF EXPERIMENTS

The results of experiments presented in this paper clearly demonstrate that the aphid *Carolinaia cyperi* can transmit mosaic from diseased to healthy plants. The aphid *Hysteroneura setariae* also was shown to be a vector of sugarcane mosaic, thus confirming the results obtained by Ingram and Summers (5) in Louisiana. These findings will undoubtedly help to explain the field spread of sugarcane mosaic, particularly under such conditions as exist in Puerto Rico.

Although *Carolinaia cyperi* does not colonize on sugarcane, its host plant *Cyperus rotundus* commonly grows in and around sugarcane fields, and it does not seem improbable that during the course of migration, resulting from such factors as overcrowding or destruction of its host plant, it may feed for short intervals on sugarcane and thus become a means of spreading mosaic.

When seedlings were used as the experimental plants, *Carolinaia cyperi* proved to be as good as, if not better than, *Aphis maidis* as a vector of mosaic, but when cuttings were used the percentage of successful transfers of the disease was considerably less than with *A. maidis*. This probably was partly attributable to the fact that *C. cyperi* fed less readily on plants grown from cuttings than on seedlings, possibly because of their comparative softness and tenderness and also because of their grasslike characteristics in the early stage of growth. *Hysteroneura setariae* proved to be a less efficient vector of sugarcane mosaic than *A. maidis* a finding which confirms the results obtained by Ingram and Summers (5).

Inasmuch as the proved vectors of sugarcane mosaic are not known to occur on sugarcane to any appreciable extent in Puerto Rico, with the exception of localized infestations of *Hysteroneura setariae*, it appears that field dissemination is largely dependent upon insects that are more or less incidentally associated with sugarcane. Evidence in support of such a contention has been presented in connection with certain other viruses. Drake, Tate, and Harris (4) found that aphids, which do not normally feed on the onion plant, are responsible for the natural spread of yellow dwarf, a virus disease of onions. Zaumeyer and Kearns (8) succeeded in transmitting bean mosaic by means of 11 species of aphids, none of which ordinarily colonize on the bean plant, and these workers concluded that other species probably would be found to act as vectors of this disease.

The percentage of successful transfers obtained in insect-transmission experiments with sugarcane mosaic has been comparatively low, and it has been necessary to use large numbers of plants to obtain conclusive results. Such a condition necessitates the use of considerable greenhouse space, which is expensive and frequently not available, for growing the plants during the observation period.

In an effort to simplify the situation, plants grown from seed of the variety SC 12 (4) were used and the results compared with those obtained from plants grown from cuttings.

Seedlings proved to have an advantage from the standpoint of convenience and rapidity of handling and economy of greenhouse space. In 1 square foot of greenhouse space 16 to 20 seedlings could be conveniently used in transmission experiments, whereas in a similar space not more than 6 to 8 plants grown from cuttings could be satisfactorily handled. Of 262 seedling plants exposed to *Carolinaia cyperi* and *Aphis maidis*, 99, or an average of 37.8 percent, developed mosaic. From 130 exposures of plants grown from cuttings 30, or 23 percent, resulted in positive transmissions. In seedlings the average incubation period of the disease in the plant was 19.3 days, and in cuttings it was 26.2 days.

The chief disadvantage in using seedlings was that plants in a satisfactory stage of growth for conducting small cage experiments were not available throughout the year.

SUMMARY

Experiments are described which demonstrate that the aphid *Carolinaia cyperi* can transmit mosaic from diseased to healthy sugarcane plants. Out of 192 plants exposed to *C. cyperi* taken from diseased plants, 60, or 31.2 percent, became diseased, and of 200 healthy plants exposed to infective *Aphis maidis*, 69, or 34.5 percent, developed mosaic.

Field observations showed that *Carolinaia cyperi* was present on its host plant, *Cyperus rotundus*, in and around sugarcane fields in Puerto Rico in considerable abundance during certain periods of the year.

Transmission experiments with *Hysteroneura setariae* convincingly demonstrated that this aphid could transmit mosaic from diseased to healthy sugarcane plants, thus confirming the evidence obtained by previous investigators.

Of a total of 75 healthy sugarcane plants exposed to *Sipha flava* taken from diseased plants, none developed mosaic.

In most cases a considerably higher percentage of transmission was obtained when seedling plants were exposed to aphids that had fed on diseased plants than when cuttings were used.

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No. 2

EFFECT OF VARIOUS GRADES OF FERTILIZERS ON THE SALT CONTENT OF THE SOIL SOLUTION¹

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INTRODUCTION

Crops grown on different soils differ greatly not only in yields but also in quality, appearance, and resistance to spoilage. Climate, water relations, biological conditions, and the physical properties of a soil have a great deal to do with its crop-producing capacity, but even with these factors at the optimum good crops cannot be obtained if the soil solution contains an excess of soluble salts or an inadequate supply of any essential plant-food element.

One of the most effective methods for controlling the composition of the soil solution is treatment with fertilizers. Soluble salts of some of the minor fertilizer elements are toxic to plants at relatively low concentrations, injuring them by changing the permeability of their cell walls or otherwise interfering with their metabolism. The ordinary fertilizer salts are less toxic to plants and give rise to crop burning only when a sufficient quantity is present to develop an osmotic pressure in the soil solution that approaches or exceeds that of the plant sap. Water then enters the plant too slowly to compensate for that lost by transpiration, or it actually passes from the roots by osmosis. In either case, the plant withers and dies.

The purpose of this investigation was to develop a laboratory method for measuring the influence of fertilizers on the concentration of the soil solution with a view to determining whether danger from salt injury is being increased or decreased by the changes taking place in the composition of fertilizers. The work was limited to a study of the ordinary fertilizer materials and mixtures which are considered to be nontoxic to plants below a concentration that produces plasmolysis in the ordinary cultivated crops. It is recognized that factors other than concentration determine the burning effects of solutions. Composition, especially the relative proportion of divalent and monovalent elements, is of particular importance. The solutions recovered in this work from soils treated with various fertilizer materials contained calcium as well as the monovalent elements resulting from base-exchange reactions. With one exception both types of elements were present in all mixtures used in the tests.

PROCEDURE

Some fertilizer materials react with the constituents of the soil to a much greater extent than others. A mixture containing a high pro-

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² The authors acknowledge their indebtedness to H. G. Byers and M. S. Anderson, Soil Chemistry and Physics Research Division, Bureau of Plant Industry, U. S. Department of Agriculture, for helpful suggestions in carrying out this investigation; and to Charles E. Kellogg, Chief, Soil Survey Division, Bureau of Plant Industry, for supplying the soils used in the experimental work.

portion of soluble salts that undergo fixation in the soil may therefore increase the concentration of the soil solution less than one containing a lower percentage of soluble salts that are not fixed in the soil. The soluble-salt content of different fertilizer mixtures cannot therefore be employed as an accurate measure of their influence on the soil solution. The method used in this investigation for determining the effect of any given fertilizer consisted in making a direct determination of the change in the effective concentration of the soil solution as a result of the fertilizer treatment.

Methods proposed for obtaining the true soil solution may be grouped into three classes, depending on whether the solution is separated from the soil by (1) application of high pressure (8)³;

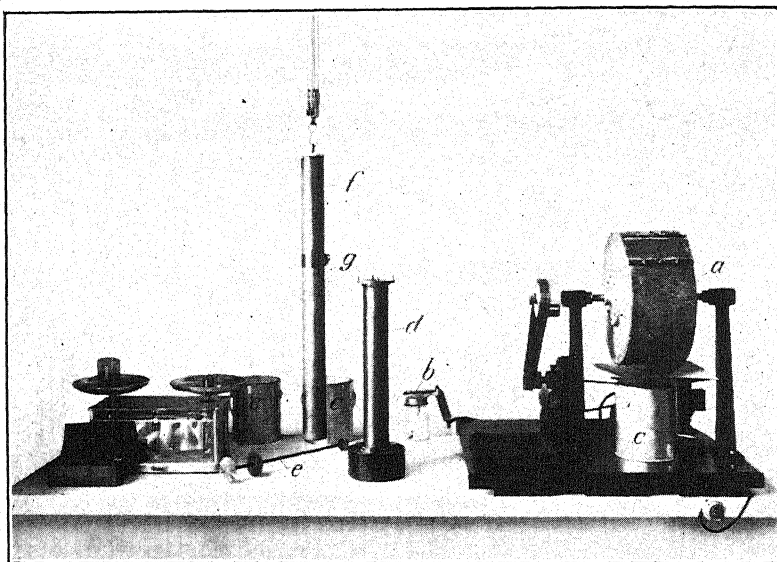


FIGURE 1.—Equipment for treating the soil preparatory to the recovery of the soil solution.

(2) centrifugal action (2); or (3) displacement with a liquid (3, 4, 6, 9, 10, 14, 15).

The displacement method has advantages over the other two methods in that it requires less complicated apparatus and permits the recovery of larger quantities of the soil solution from a soil of given moisture content. It was first developed by Schloesing (14), who used water colored with carmine to displace the soil solution. Ishcherekov (6) used ethyl alcohol as the displacing agent and obtained results which indicated that the displaced solution is the true soil solution. Parker (10) showed that the use of such different displacing agents as acetone, methyl alcohol, water, and ethyl alcohol has little or no effect on the concentration of the recovered soil solution and that the displacement method is well adapted to "a study of the composition and reaction of the soil solution under any condition."

That the composition of the solution recovered by displacement approximates that of the true soil solution was further demonstrated

³ Italic numbers in parentheses refer to Literature Cited, p. 98.

by Burd and Martin (3), who used the displaced soil solution from a portion of soil as the displacing medium for a second portion of the same soil. The newly displaced solution had the same concentration as the displacing solution, indicating that this displaced solution had the same concentration as the solution with which it came in contact in the soil. It was also shown by Burd and Martin that the time of recovering the displaced solution could be shortened and the yield of solution by water displacement increased by applying air pressure to the closely packed soil in a closed container. Accordingly, the displacement method was adopted for the recovery of the soil solution in this investigation.

The fertilizer to be tested was mixed with the soil in an air-dried condition. The soil was then sprayed with sufficient water to bring

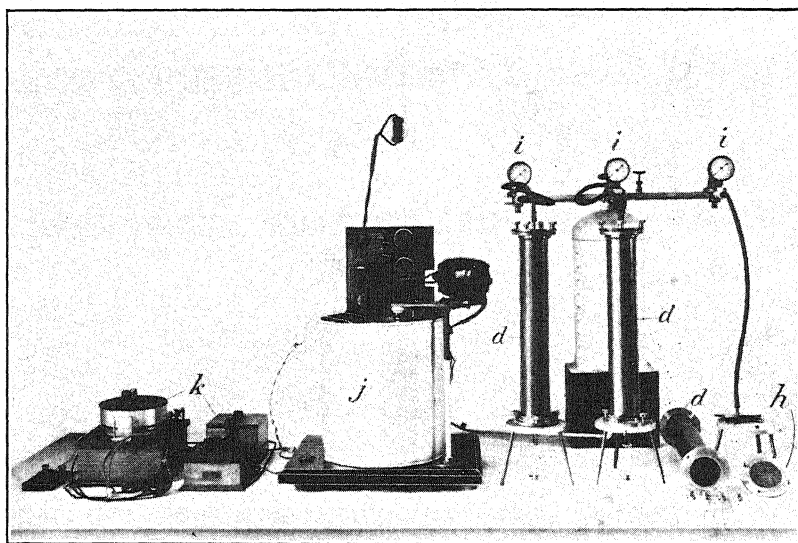


FIGURE 2.—Equipment for displacing the soil solution and measuring its specific conductivity.

the moisture content to 75 percent of its moisture equivalent (1, 2), after which it was stored in a closed container until it reached a state of equilibrium with respect to the added moisture and fertilizer. A comparison of the concentration of soil solutions from samples stored for 1, 3, 5, and 30 days showed that storage for 5 days is ample for this purpose. In most of the tests the samples were maintained at 5° C. during storage with a view to avoiding appreciable change in the composition of the soil solution as a result of biological action. The soil solution was finally displaced from the soil, and the effect of the added fertilizer was determined by comparing the concentration of the recovered solution with that of the soil solution displaced from the unfertilized soil.

The equipment used in the work is shown in figures 1 and 2.

Figure 1 shows a device (*a*) for mixing the fertilizer with the dry soil, a spray gun (*b*) for spraying the water on the soil while it is being rolled on a rubber sheet, friction-top cans (*c*) for storing the soil, and equip-

ment for packing the stored soil into the displacement cylinders (*d*). The soil was transferred to the cylinders in increments of 150 gm. The surface of the soil in the cylinder was leveled off with the instrument (*e*), and each addition of soil was then

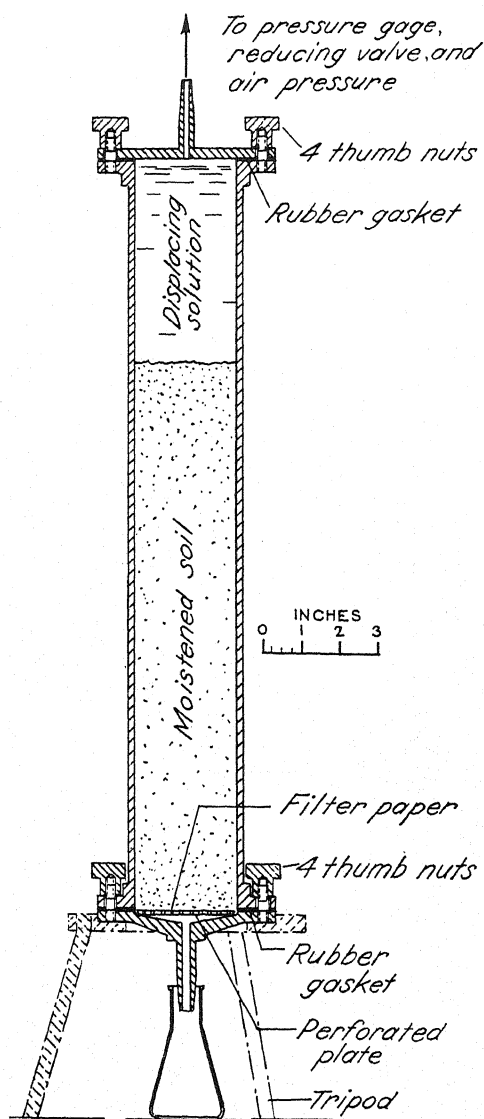


FIGURE 3.—Brass cylinder for displacing soil solutions.

packed in the cylinder by allowing the plunger (*f*) to fall three times from a height of 12 cm., as measured by the chain (*g*). The soils were packed to a degree that permitted ready flow of the displaced solution without excessive dilution by the displacing medium because of too rapid flow or channeling. A 40-pound plunger gave the best results with the Norfolk sandy loam soil used in the tests and a 20-pound plunger with the Cecil clay loam soil.

Figure 2 shows the reducing valves and gages (*i*) for controlling and measuring the pressure applied to each cylinder, the constant temperature bath (*j*) in which cells *C* and *D* (fig. 4) were supported to bring the solution to the desired temperature (30° C.) for measurement, and the electrical equipment (*k*) used in making the conductivity measurements.

Each cylinder, shown assembled in figure 2 and diagrammatically in figure 3, was 7 cm. in diameter and 45 cm. long, constructed of brass throughout, and provided with a perforated bottom (fig. 2, *h*), over which a filter paper was placed to support the soil.

The liquid used as displacing agent was poured over the soil in the cylinder to a depth of about 12 cm. The top of the cylinder was then closed, and after 1 hour sufficient air pressure was applied to cause the displaced soil solution to drop rapidly from the cylinder. A clear solution was recovered from both soils used in the tests except

when treated with excessive applications of fertilizers or with a basic material such as free ammonia.

Distilled water was used as the displacing agent for soils receiving a large application of fertilizer, while 0.1 normal sodium chloride solution was used as the displacing agent for soils having a low content of soluble salts. In this way a marked difference was always maintained between the concentration of the soil solution and that of the displacing agent, and the point at which further recovery of the solution would mean contamination with the displacing agent could be readily determined by making conductivity determinations of successive 5- or 10-cc. portions of the recovered solution.

The types of conductivity cells used in the work are shown in figure 4. Conductivity cells *A* and *B* were used in making quick tests

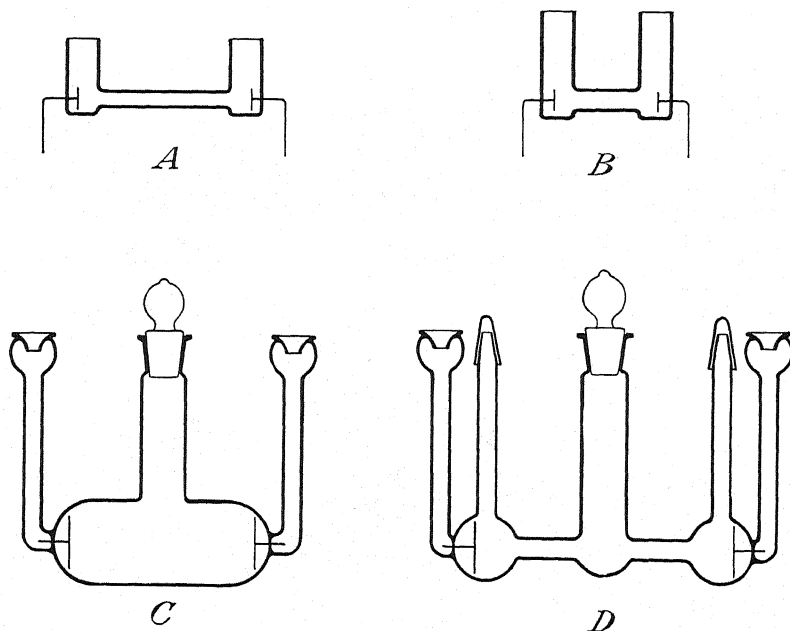


FIGURE 4.—Conductivity cells.

for any change in the concentration of the recovered soil solution, while cells *C* and *D* were used in measuring the relative concentrations of different soil solutions by a determination of their specific conductivities. Conductivity cell *C* was adapted for measuring the concentration of solutions having a specific conductivity below $2 \times 10^{-3} \text{ ohm}^{-1}$, while cell *D* was used for solutions having a specific conductivity in excess of this value. The constants of cells *C* and *D* were determined by the method of Parker and Parker (11).

Specific conductivity measurements are adapted for determining the relative concentrations of solutions containing the same or similar combinations of inorganic salts, but freezing-point measurements give more accurate results for the effective concentrations of solutions that contain organic materials. As a check on the results both methods were used for all solutions, but the results with both methods are

shown only in figure 12. In all the other figures in which curves are shown the variation in the effective concentration of the soil solution with increased application of a fertilizer is expressed in terms of its osmotic pressure, as calculated from the freezing-point depression.

The osmotic pressure P , in atmospheres, of an aqueous solution at its freezing point may be calculated (?) from the difference Δ , in degrees centigrade, between its freezing point and that of pure water by the equation

$$P = 12.06\Delta - 0.021\Delta^2 \text{ ----- (1)}$$

Values of P in this equation may be conveniently obtained from the table of Harris and Gortner (5) for all values of Δ between 0.001° and 2.999° C. All freezing-point determinations were made by the Beckmann method, which has an accuracy of $\pm 0.005^\circ$ (3).

SOILS USED IN TESTS

Two soils were used in the tests: A Norfolk sandy loam of low fixing power from Edgefield County, S. C., and a Cecil clay loam of high fixing power from Abbeville County, S. C. Before it was used, each soil was air-dried and passed through a 10-mesh screen. The mechanical analyses of the two soils are given in table 1.

TABLE 1.—Mechanical analysis of soils¹

Type of soil	Fine gravel	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt	Clay	Colloid (included in clay)
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Norfolk sandy loam.....	6.0	25.2	15.8	24.8	13.2	8.5	6.3	4.2
Cecil clay loam.....	1.3	3.3	3.9	16.1	11.7	16.2	47.2	41.3

¹ Analysis by T. M. Shaw, Soil Chemistry and Physics Research Division, Bureau of Plant Industry.

The pH value of the Norfolk soil used in this investigation was 5.29 as determined by the hydrogen electrode, and that of the Cecil soil was 5.20. The Norfolk soil had a moisture equivalent of 5.1 percent; that of the Cecil soil was 26.3 percent. The volume of soil solution in 1,000 gm. of the Norfolk soil when its moisture content was adjusted to 75 percent of its moisture equivalent was therefore 36.6 cc., while the corresponding volume of soil solution in the same weight of Cecil soil was 164.9 cc.

EXPERIMENTAL RESULTS

INFLUENCE OF DIFFERENT FERTILIZER MATERIALS ON THE CONCENTRATION OF THE SOIL SOLUTION

All fertilizer materials used directly in the tests were of c. p. grade with the exception of kainit, manure salts, cottonseed meal, and superphosphate. The materials used in the preparation of the fertilizer mixtures were of commercial grade. In calculating rates of application it was assumed that the surface 6-inch layer of an acre of dry soil weighs 2,000,000 pounds. The specific conductivities of the solutions from samples of a soil receiving the same fertilizer treatment usually agreed within 3 percent. Duplicate determinations that varied more than 5 percent were repeated.

The curves in figure 5 show the results obtained for the osmotic pressures of the solutions displaced from the Norfolk soil treated with increasing applications of various fertilizer materials. The curves show that for equal applications of plant food the phosphates and free ammonia have the least effect on the salt content of the soil solution, while sodium nitrate and the low-grade potash salts have the greatest effect. The order of these materials remained the same

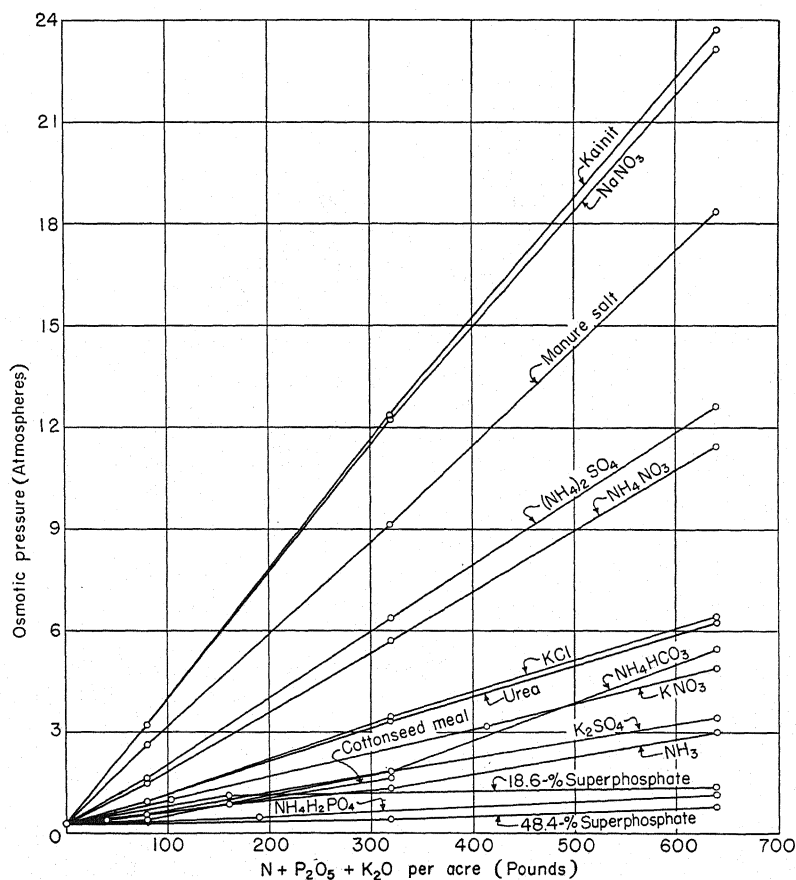


FIGURE 5.—Influence of various fertilizer materials on the osmotic pressure of the soil solution from Norfolk sandy loam soil.

when the tests were made with the Cecil soil. Long experience with several of these materials shows that they fall in the same order under field conditions, indicating that the laboratory method gives a true measure of the relative influence of fertilizers on the concentration of the soil solution.

The relative effects of increasing applications of different nitrogenous materials on the concentration of the soil solution in Norfolk soil are indicated in figure 6. The curves in this figure show that the materials fall in the same order for all applications up to the maximum

used of 640 pounds of nitrogen per acre.⁴ This order remained substantially the same, as shown in figure 7, when the materials were applied to the Cecil soil. A comparison of the curves in the two figures shows, however, that when the moisture in each soil was adjusted to three-fourths of its moisture equivalent the same application of fertilizer increased the concentration of the soil solution in the Norfolk soil to a much greater extent than in the Cecil soil.

The effect of urea on the concentration of the soil solution in soils stored at 5° C. was usually between that of ammonium nitrate and ammonium bicarbonate (figs. 5 and 6) for equal applications of nitrogen. At summer temperatures urea rapidly decomposes in the soil

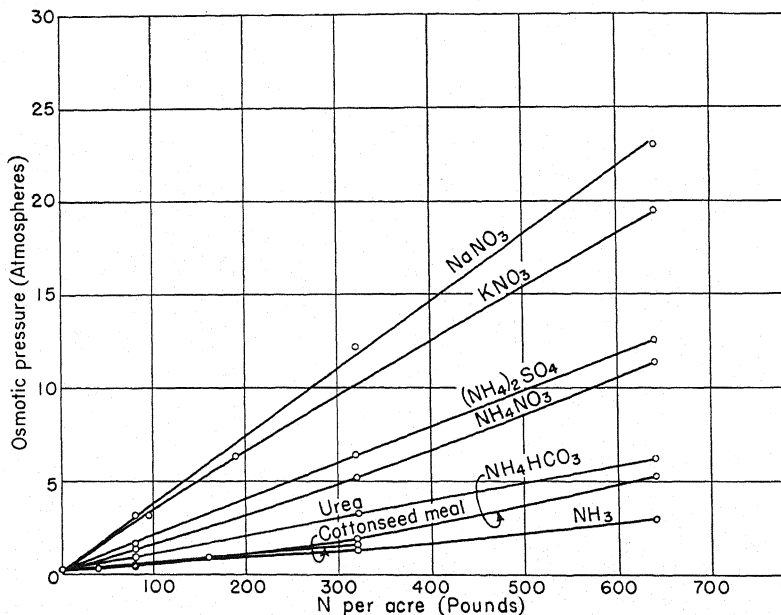


FIGURE 6.—Influence of various nitrogenous materials on the osmotic pressure of the soil solution from Norfolk sandy loam soil.

to form ammonia and ammonium bicarbonate (12). This decomposition of one mole of urea to form one mole each of two decomposition products would normally tend to increase the concentration of the soil solution, but owing to the relatively high fixing power of soils for ammonia the effect of urea on the concentration of the soil solution was actually found to be less at 25° than at 5°.

The striking difference in the effects of different potash salts on the concentration of the soil solution is shown in figures 8 and 9. The curves show, for example, that 80 pounds of potash as high-grade kainit (20 percent K₂O) has about the same effect on the concentration of the soil solution as 640 pounds of potash as potassium sulphate. The kainit used in the tests contained about 60 percent of sodium

⁴ An application of 640 pounds of nitrogen per acre, or a comparable amount of P₂O₅ or K₂O, is much greater than that ordinarily applied under field conditions. Applications up to this maximum were considered advisable in this investigation in order to reproduce the concentrations of salts found in the seed zone when fertilizers are applied in bands at the side of the seed or plant as now recommended for most row crops (15).

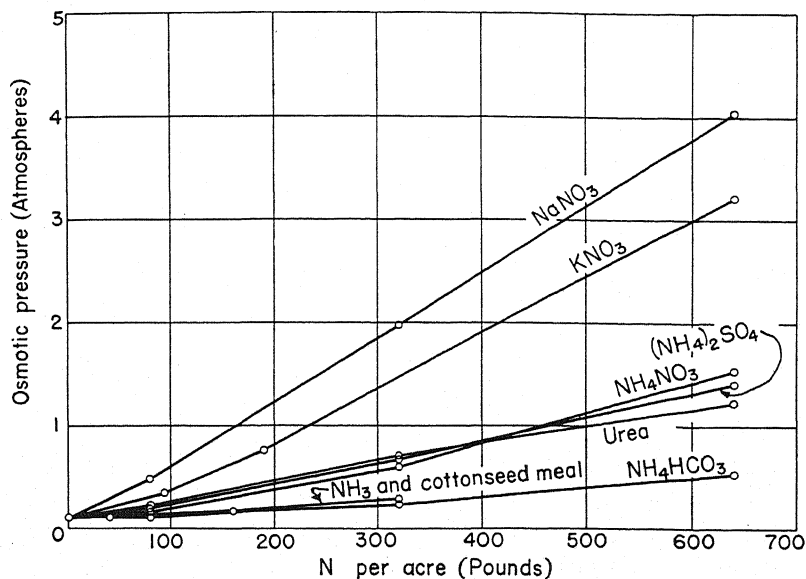


FIGURE 7.—Influence of various nitrogenous materials on the osmotic pressure of the soil solution from Cecil clay loam soil.

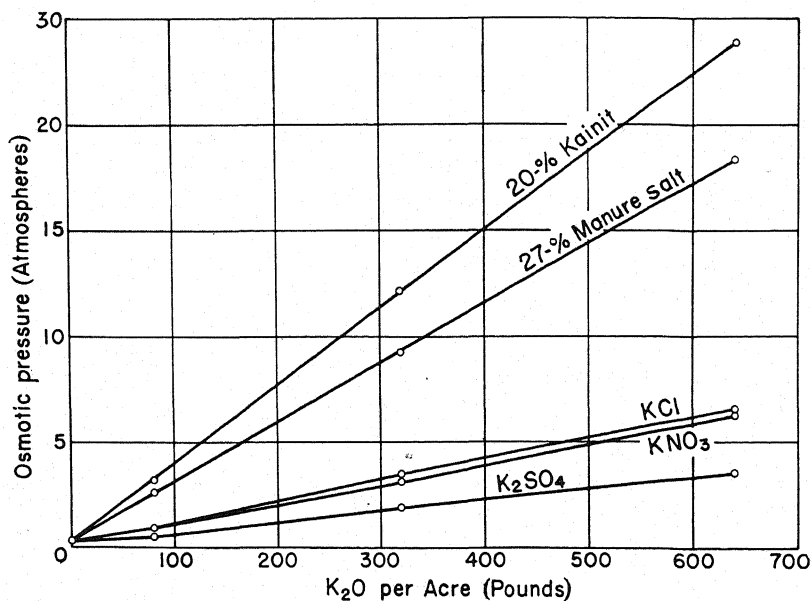


FIGURE 8.—Influence of various potash salts on the osmotic pressure of the soil solution from Norfolk sandy loam soil.

chloride in addition to potassium chloride and smaller amounts of other salts. As the grade of the kainit decreases the proportion of sodium chloride increases. Sodium chloride contains none of the primary fertilizer elements, but it produces plant burning as readily as high-grade potassium chloride containing upward of 60 percent of plant food. If low-grade rather than high-grade kainit had been used in the tests, the differences cited would have been still more striking. The potash salts fall in the same order for the Norfolk and Cecil soils when arranged according to their effect on the soil solution. This arrangement in ascending order is potassium sulphate, potassium nitrate, potassium chloride, manure salts, and kainit.

That superphosphate and monoammonium phosphate have a relatively small effect on the salt content of soil solutions even for appli-

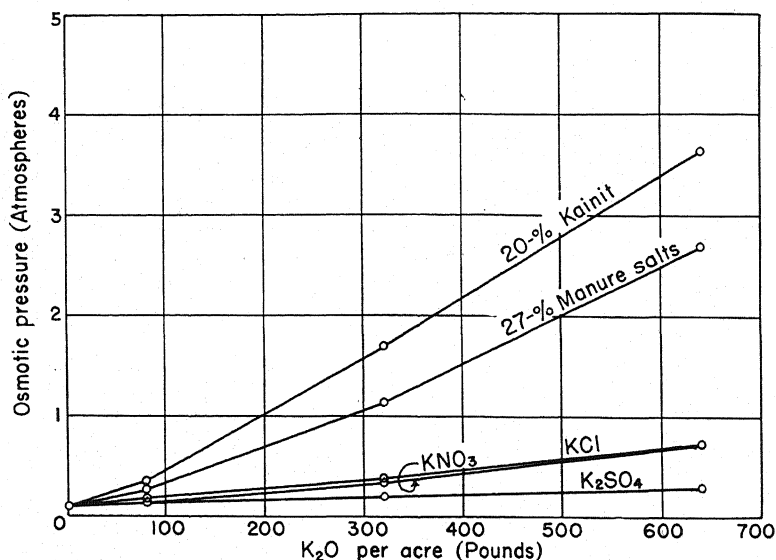


FIGURE 9.—Influence of various potash salts on the osmotic pressure of the soil solution from Cecil clay loam soil.

cations up to 1,280 pounds of P_2O_5 per acre is shown by the curves in figure 10. A direct comparison of superphosphate with sodium nitrate shows that an application to Norfolk soil of 6,000 pounds of the former has no greater effect on the concentration of the soil solution than 250 pounds of the latter.

According to Parker (10), the concentration of a soil solution is inversely proportional to the moisture content of the soil, and while the product of the freezing-point lowering of the soil solution and of the moisture content of a soil differs for different soils and for varying applications of a fertilizer on the same soil, it is fairly constant within limits for any given soil treatment. The value of this constant, K , may therefore be calculated from the equation $K = \Delta M$, where Δ is the freezing-point lowering of the solution displaced from the soil and M is the moisture content of the soil. Knowing the value of K as determined for a soil of a given moisture content, the approximate

value for the osmotic pressure of the soil solution in the soil at a different moisture content may then be readily calculated by substituting K/M for Δ in equation 1.

TABLE 2.—*Osmotic pressures of the soil solutions from fertilized and unfertilized Norfolk and Cecil soils of the same moisture content*

Fertilizer treatment		Osmotic pressure of—		
		Norfolk soil solution		Cecil soil solution, found for moisture =19.7 percent
Material	Plant food per acre	Found for moisture =3.8 percent	Calculated for moisture =19.7 percent	
	<i>Pounds N</i>	<i>Atmospheres</i>	<i>Atmospheres</i>	<i>Atmospheres</i>
None.....	None	0.25	0.05	0.11
Ammonium sulphate.....	80	1.63	.31	.20
Do.....	320	6.35	1.23	.66
Do.....	640	12.60	2.44	1.39
Sodium nitrate.....	80	3.22	.63	.48
Do.....	320	12.26	2.38	1.95
Do.....	640	23.20	4.48	4.10
Ammonium nitrate.....	80	1.47	.25	.19
Do.....	320	5.64	1.09	.60
Do.....	640	11.44	2.21	1.52
Ammonia.....	80	.54	.11	.12
Do.....	320	1.33	.25	.28
	<i>K₂O</i>			
Potassium chloride.....	80	.93	.18	.17
Do.....	320	3.44	.66	.37
Do.....	640	6.54	1.27	.76
Potassium sulphate.....	80	.51	.09	.15
Do.....	320	1.82	.35	.21
Do.....	640	3.47	.66	.30
Potassium nitrate.....	80	.93	.18	.13
Do.....	320	3.18	.62	.35
Do.....	640	6.27	1.21	.75
Kainit, 20 percent K ₂ O.....	80	3.21	.62	.35
Do.....	320	12.36	2.39	1.71
Do.....	640	23.80	4.60	3.86
	<i>P₂O₅</i>			
Monoammonium phosphate.....	160	.45	.08	.16
Do.....	640	1.32	.25	.29
Do.....	1,280	3.83	.74	.45
Superphosphate.....	160	1.10	.22	.19
Do.....	640	1.38	.27	.64
Do.....	1,280	1.98	.39	1.09
Double superphosphate.....	160	.42	.08	.14
Do.....	640	.78	.16	.27
Do.....	1,280	1.74	.34	.37

In table 2 are given the osmotic pressures found for the soil solutions displaced from Norfolk and Cecil soils with moisture contents of 3.8 and 19.7 percent, respectively, or three-fourths of their water equivalents, and the calculated osmotic pressures of the corresponding soil solutions from the Norfolk soil on the basis of a moisture content of 19.7 percent, or the same as that of the Cecil soil.

The results given in table 2 show that the differences in the osmotic pressure of the soil solution of Norfolk and of Cecil soils are relatively small when their moisture content is the same. It would seem, therefore, that the difference in the effect of any given fertilizer on the concentration of the soil solution in these two soils when the moisture

content of each is adjusted to three-fourths of its water equivalent is due mainly to the difference in their moisture content rather than to any inherent difference in their chemical and physical properties. The results further show that while a given application of fertilizer increases the concentration of the soil solution in Norfolk soil to a much greater extent than in Cecil soil (figs. 6 to 10) when the moisture in each soil is at the optimum for crop growth, the reverse may be true if the Cecil soil is dry and the Norfolk soil is relatively wet.

A comparison of the last two columns of table 2 shows that the osmotic pressure of the soil solution in the Norfolk soil at a moisture content of 19.7 percent is usually greater than that of the corresponding solution from the Cecil soil of the same moisture content, and that these differences increase with increase in the fertilizer application.

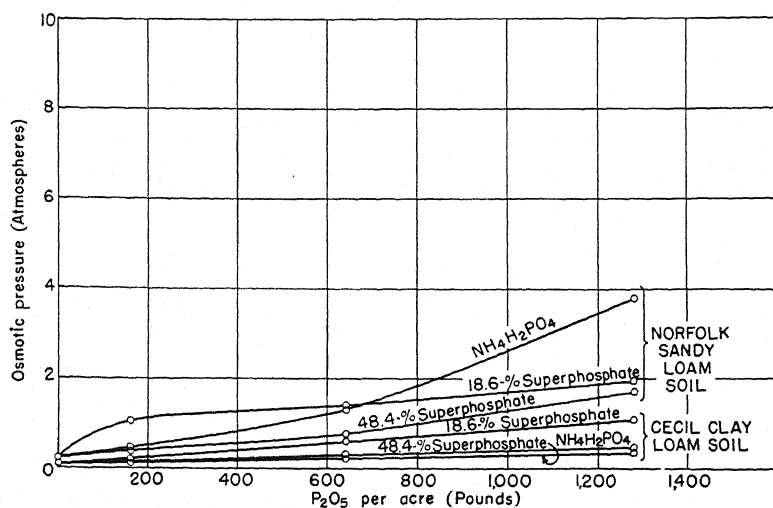


FIGURE 10.—Influence of various phosphatic materials on the osmotic pressure of the soil solution from two soils.

Although these differences are relatively small, as already pointed out, they appear to be larger than the limit of experimental error and indicate a greater fixation of fertilizer in the Cecil than in the Norfolk soil of the same moisture content.

INFLUENCE OF MIXED FERTILIZERS ON THE CONCENTRATION OF THE SOIL SOLUTION

The fertilizer formulas given below are typical of mixtures in use at different periods in the history of the fertilizer industry (13).

Material:	Year 1880; Grade, 2-9-2	Pounds per ton
Fish scrap, 6.0 percent N; 8.0 percent P ₂ O ₅	600	
Sodium nitrate, 15.5 percent N.....	27	
Superphosphate, 12.5 percent P ₂ O ₅	1, 053	
Kainit, ¹ 12.5 percent K ₂ O.....	320	
Total.....	2, 000	

¹Approximate percentage composition: KCl, 20; NaCl, 75; sulphates, 1; insoluble matter, 4.

*Year 1910; Grade, 3-9-3**Material—Continued.*

Ammonium sulphate, 20.0 percent N.....	100
Sodium nitrate, 15.5 percent N.....	130
Cottonseed meal, 7.0 percent N.....	285
Superphosphate, 16.0 percent P_2O_5	1, 125
Manure salts, ² 20.0 percent K_2O	300
Filler.....	60
Total.....	2, 000

Year 1937; Grade, 4-9-5

Ammonium sulphate, 20.5 percent N.....	166
Ammonia, 82.3 percent N.....	21
Sodium nitrate, 16.0 percent N.....	63
Tankage, 7.0 percent N.....	170
Urea, 46.6 percent N.....	18
Superphosphate, 19.0 percent P_2O_5	947
Potassium chloride, ³ 50.0 percent K_2O	200
Dolomite.....	224
Filler.....	191
Total.....	2, 000

² Approximate percentage composition: KCl, 32; NaCl, 61; sulphates, 1; insoluble matter, 6.

³ Approximate percentage composition: KCl, 79; NaCl, 4; insoluble matter, 17.

The first of the three formulas given shows that the average mixture consumed in 1880 contained about 2 percent of N, 9 percent of P_2O_5 , and 2 percent of K_2O , or a total of 13 percent of plant food. Organic nitrogen was the cheapest form of nitrogen at that time, and the organic ammoniates were therefore the principal nitrogenous materials used in fertilizer mixtures. The spread in the cost of the different forms of nitrogen soon disappeared, however, and for a period centering around 1890 the cost of nitrogen remained about the same for the different carriers in which it appeared on the market. Consequently, the practice was developed of using equal quantities of all three forms of nitrogen in the preparation of mixed fertilizers. This practice was continued through 1910, although by that time the cost of organic nitrogen had become greater than that of either of the other two forms.

The third formula, above, shows that the typical present-day mixture differs from that in use in either 1880 or 1910 in that it contains a fourth form of nitrogen (amide nitrogen) as Cyanamid or urea. Ammonia nitrogen is now the cheapest form of nitrogen, and its use in mixed fertilizers, as free ammonia, ammonium nitrate, and ammonium sulphate, exceeds by more than 50 percent that of all other forms of nitrogen combined. Dolomite is also used in mixed fertilizers to correct their acid influence on the soil. It will be noted that during the history of the industry the P_2O_5 content of the superphosphate used in mixed fertilizers has increased from 12.5 to 19.0 percent, the K_2O content of the potash salts from 12.5 to 50.0 percent, and the total plant-food content of fertilizer mixtures from 13.0 to 18.0 percent. These formulas show that a marked change has taken place in the grade as well as in the composition of fertilizer mixtures.

The extent to which these changes in the grade and composition of fertilizers has influenced their effect on the concentration of the soil solution is shown in figure 11. The curves show that the mixtures of 1880 and 1910 are much alike in their effect on the salt content of the

soil solution in both Norfolk and Cecil soils, whereas the influence of the present-day mixture is considerably less than that of either of the other two. This indicates that the danger from salt injury to plants by fertilizers is decreasing rather than increasing, as might be supposed from the increased use of the synthetic products in mixed fertilizers. The principal factors that contribute to the decreased salt effect of the average present-day mixtures are (1) the increased plant-food content of the mixture whereby less fertilizer has to be distributed per acre for a given application of plant food, (2) the replacement of kainit and other low-grade potash salts with high-grade

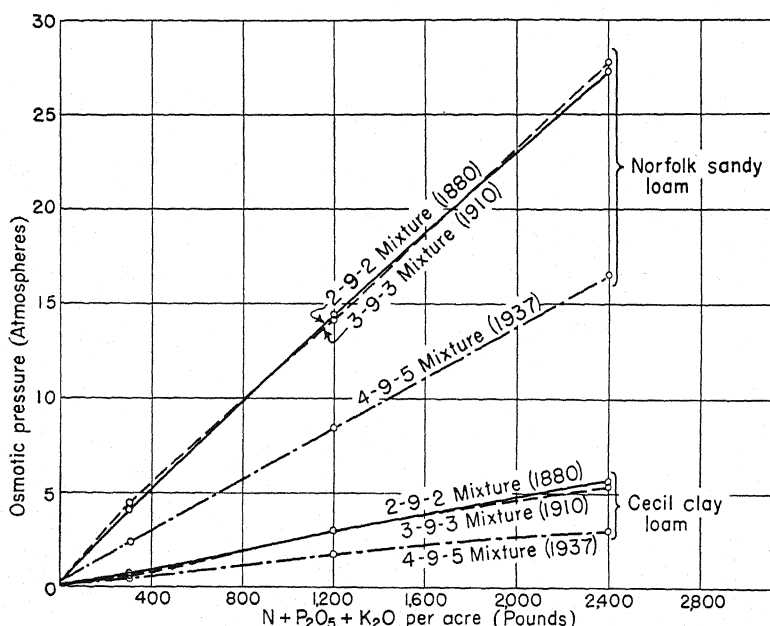


FIGURE 11.—Relative influence of typical present-day mixed fertilizers on the osmotic pressure of the soil solution in two soils.

muriate, and (3) the substitution in part of free ammonia for other soluble nitrogenous materials. These changes, as indicated by the curves in figures 6, 7, 8, and 9, have a marked effect in decreasing the influence of fertilizers on the salt content of the soil solution.

Formulas of three other fertilizer mixtures that have been or are now in general use are given in table 3. A fertilizer mixture prepared according to the 3-8-4 formula given in the table contains 400 pounds of manure salts per ton and is typical of mixtures in use about 15 years ago. In fertilizing cotton it was common practice to apply a mixture of this kind to the soil a week or so in advance of planting and then a like quantity of nitrogen later as a side dressing. If both fertilizer treatments had been made at the same time, the application would have been equivalent to that of a 6-8-4 fertilizer. The materials used in the 6-8-4 mixture (table 3) are representative of those in use at present. The materials composing the double-strength 6-16-8

mixture are also typical of those now in use. The results obtained with these three mixtures are shown in figure 12. The solid-line curves represent the changes in the specific conductivity, in reciprocal ohms, of the soil solution in the Norfolk and Cecil soils with increased applications of fertilizer, and the broken-line curves represent the changes in the osmotic pressure of the solutions. The two sets of curves show a considerable spread with increased application of the fertilizer, particularly on the Norfolk soil. This is due, as already

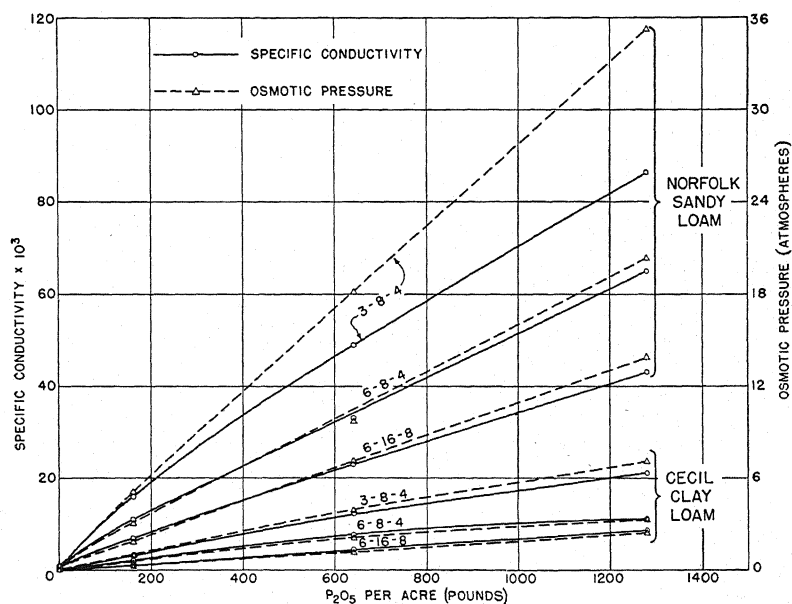


FIGURE 12.—Influence of mixed fertilizers of different types on the concentration of the soil solution in two soils.

explained, to the solvent action of high concentrations of certain salts on the organic matter of the soil.

TABLE 3.—*Formulas of fertilizer mixtures of different types*

Material	Quantity per ton in mixed fertilizer—		
	3-8-4	6-8-4	6-16-8
	Pounds	Pounds	Pounds
Ammonium sulfate, 20.8 percent N.....	96	252	105
Ammonium nitrate, 35.0 percent N.....		70	119
Ammonia, 82.3 percent N.....		23	40
Sodium nitrate, 16.3 percent N.....	123		
Cottonseed meal, 7.0 percent N.....	286	343	343
Superphosphate, 16.0 percent P ₂ O ₅	1,000		
Superphosphate, 20.6 percent P ₂ O ₅		777	294
Double superphosphate, 48.8 percent P ₂ O ₅			535
Manure salts, 20.0 percent K ₂ O.....	400		
Potassium chloride, 60.1 percent K ₂ O.....		133	266
Dolomite.....		402	298
Filler (sand).....	95		
Total.....	2,000	2,000	2,000

The curves of figure 12 also show that the double-strength 6-16-8 mixture has less effect on both the conductivity and osmotic pressure of the soil solution than the 6-8-4 mixture and that the effect of this mixture is less than that of the single-strength 3-8-4 mixture in use about 15 years ago. This relationship holds true for both soils and for all applications up to the maximum of 1,280 pounds of P_2O_5 per acre, equivalent to 8 tons of the 6-8-4 mixture or 4 tons of the 6-16-8 mixture. Such high applications are never made broadcast, but in band placements concentrations of this order may often be approached in the vicinity of the seed zone.

It should be emphasized that while a double-strength mixture prepared according to the formula of table 3 has less effect on the

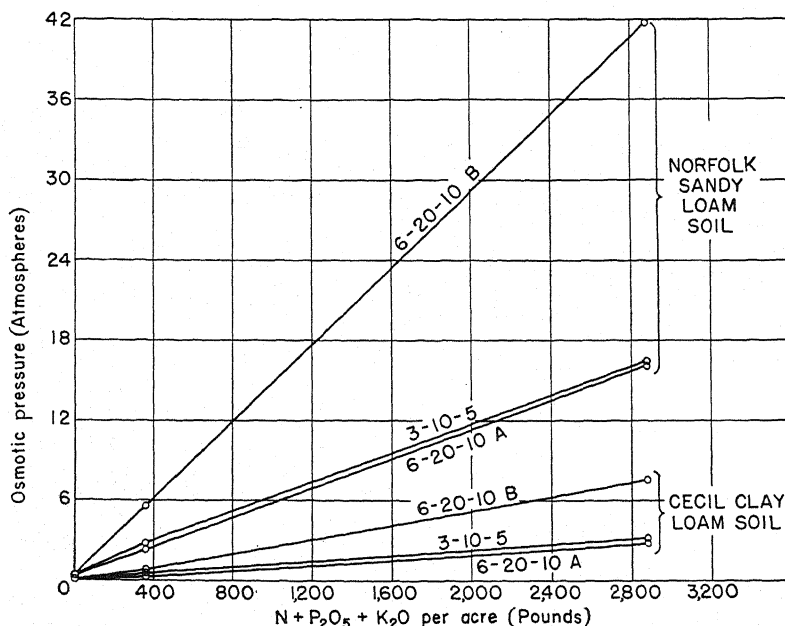


FIGURE 13.—Relative influence of single- and double-strength mixed fertilizers on the osmotic pressure of the soil solution in two different soils.

concentration of the soil solution than the corresponding single-strength mixture, this need not necessarily hold for all types of mixtures. Thus the curves in figure 13 show that the double-strength 6-20-10 mixture prepared according to formula A of table 4 has less effect on the concentration of the soil solution of the soils used in the tests than the corresponding single-strength 3-10-5 mixture, but the reverse is true when the double-strength mixture consists of Ammophos, sodium nitrate, and low-grade potash salts, as specified in formula B of table 4. Ammophos, sodium nitrate, and the different grades of potash salts are extensively used in fertilizer mixtures, but a mixture prepared from these materials only does not contain an adequate proportion of the secondary plant-food elements, and it is therefore not to be recommended for continued use on soils deficient in these elements.

TABLE 4.—*Formulas of single- and double-strength fertilizer mixtures*

Material	Quantity per ton in mixed fertilizer—		
	3-10-5	6-20-10	
		A	B
	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Ammonium sulfate, 20.8 percent N.....	100	200	-----
Ammonia, 82.3 percent N.....	30	60	-----
Ammophos A, 10.7 percent N; 48.0 percent P_2O_5	-----	-----	833
Sodium nitrate, 16.3 percent N.....	49	98	190
Cottonseed meal, 7.0 percent N.....	100	200	-----
Superphosphate, 20.4 percent P_2O_5	981	-----	-----
Double superphosphate, 46.7 percent P_2O_5	-----	858	-----
Potassium chloride, 60.2 percent K_2O	166	332	-----
Manure salts, 20.5 percent K_2O	-----	-----	977
Dolomite.....	126	252	-----
Filler (sand).....	448	-----	-----
Total.....	2, 000	2, 000	2, 000

It should be further emphasized that the relationship usually found between the total plant-food content of typical fertilizer mixtures and their effect on the soil solution does not necessarily hold true if the ratios of the plant-food elements in the mixtures are not the same or if the plant-food content is increased without corresponding decrease in the rate of application to the soil. Thus, the danger from burning for a given application of a 4-8-8 fertilizer mixture would be greater than for the same application of a 4-8-4 fertilizer of similar composition, but this would not hold true for half the application of the corresponding double-strength or 8-16-8 mixture.

It may therefore be concluded (1) that the effect of fertilizers, prepared according to present commercial practice, on the salt content of the soil solution decreases as a rule with increase in the plant-food content of the mixture when the ratio of plant food remains the same, and (2) that danger of salt injury in the use of typical present-day mixtures is less than that from mixtures formerly used, even when the nitrogen in the older type mixtures is applied to the crop in split applications.

SUMMARY

A study was made of the effect of fertilizers on the concentration of the soil solution in Norfolk sandy loam and Cecil clay loam soils. The procedure followed consisted of mixing the fertilizer to be tested with a sample of soil, adjusting the moisture content of the soil to 75 percent of its moisture equivalent, allowing it to stand at 5° C. for 5 days, separating the soil solution from the soil by the displacement method, determining the concentration of the solution by freezing-point depression measurements, and comparing its concentration with that of the solution recovered from the unfertilized soil.

For equal applications of plant food the phosphates and free ammonia had the least effect on the concentration of the soil solution of both the soils used in the tests, while sodium nitrate and the low-grade potash salts had the greatest effect.

The extent to which a fertilizer increased the concentration of the soil solution was much greater with the Norfolk soil than with the

Cecil soil. The difference in the osmotic pressure of the soil solutions of the two soils largely disappeared, however, when their moisture content was the same.

Comparison of the formulas of fertilizer mixtures representative of those in use at different periods in the history of the fertilizer industry shows that a marked change has taken place not only in the grade but also in the composition of mixed fertilizers.

Mixtures typical of those in use in 1880 and in 1910 are much alike in their effect on the concentration of the soil solution, whereas the influence of the average present-day mixture is considerably less than that of either of the other two mixtures. The reduced effect of present-day mixtures on the salt content of the soil solution is due (1) to the increased plant-food content of the mixture whereby less fertilizer has to be distributed per acre for a given application of plant food, (2) to the replacement of kainit and other low-grade potash salts with high-grade muriate, and (3) to the substitution in part of free ammonia for other soluble nitrogenous materials.

While the effect of fertilizers on the concentration of the soil solution does not necessarily decrease with increase in the grade of the mixture, this relationship holds true, as a rule, for mixtures of the same plant-food ratio when these are prepared as in present commercial practice. It is possible to prepare a 6-8-4 mixture from present-day materials that has less effect on the salt content of the soil solution than the 3-8-4 mixtures formerly used. The results indicate that danger of salt injury from typical present-day mixtures is less than that from mixtures formerly used, even when the nitrogen in the latter mixtures is applied to the crop in split applications.

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A THREE-DIMENSIONAL LATTICE DESIGN FOR STUDIES IN FOREST GENETICS¹

By BESSE B. DAY, *associate statistician*, and LLOYD AUSTIN, *in charge*, *Institute of Forest Genetics, Forest Service, United States Department of Agriculture*²

INTRODUCTION

The logical procedure for the improvement of all wild stock of any kind, whether plant or animal, is practically the same: segregation of varieties, races, and strains of the wild population; the evaluation of the characteristics of each group; the selection of the best individuals from each of the best strains; utilization of these in breeding and selection; and finally the production of new types (5).³

In forest genetics, both in breeding and in mass reforestation, the initial step involves seed selection. It is evident that there are many valuable inherent characteristics in forest trees. These need to be discovered, isolated, and defined. The measure of heredity is to be found in the offspring. Seeds from individual seed trees must be collected and sown and the behavior of the progeny therefrom studied. This necessitates the making of fairly extensive progeny tests.

The same difficulty arises in individual seed-tree progeny studies as in other plant research in varietal testing—the lack of homogeneity of the medium, soil, in which such tests are to be made. It is a well-established fact that there is much variation even in soil which seemingly has the most constant texture and quality and that the variation is reflected in the growth of the plants to such an extent that variety difference may be so completely obscured as to be lost entirely. It is only when the area is exceedingly small that soil effects may be ignored. As the number of varieties to be tested increases, the area necessary for a complete set becomes increasingly larger and the variation of soil and other growing factors are likewise magnified, resulting in what may be a considerable lack of precision. All attempts to solve this problem proved unsatisfactory for one reason or another until Yates (7, 8, 10) conceived the idea of arranging the varieties in a series of small blocks, instead of the previous arrangement in one block, distributing them in such a way that a variety variance could be calculated which would be freed of block effects. This would yield an error variance appropriate for making significance tests of differences found in the measurements of the varieties of progenies. Designs on this principle, termed “quasi-factorial” and “incomplete randomized blocks,” are readily adaptable to all phases of agronomy where varietal tests are made.

The theoretical aspects of this type of design have been treated in earlier writings (3, 7, 10). It is believed, however, that the application of this design to a particular field problem will be of interest in

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² Grateful acknowledgment is due Prof. R. A. Fisher, Galton Laboratory, University College, London, for proposing this type of design for the experiment; to F. Yates, chief statistician, Rothamsted Experimental Station, for invaluable counsel and interest in the development of this experiment; and to Prof. P. M. Barr, Division of Forestry, University of California, who made numerous suggestions leading to a more precise presentation.

³ Italic numbers in parentheses refer to Literature Cited, p. 118.

that it affords an exact test of the effectiveness of the design in ironing out heterogeneity. For this purpose the description is here given of such a design as applied to the progeny test nursery planted in April 1937 at the Institute of Forest Genetics, Placerville, Calif.,⁴ and the procedure that was followed for correcting the resulting data and making tests of significance of differences. Actual data are employed and the appropriate statistical procedure has been carried through on germination counts. In these data factors known to produce variability of conditions were actually introduced, as in the watering of sections of the experiment at unequal intervals. Their influence was reflected in the results. With the application of the correction procedure such effects were eliminated.

The objectives of the 1937 tests were as follows: (1) To determine the hereditary nature of the numerous local strains of ponderosa pine occurring in the various localities through El Dorado County, Calif., including probable casual relationships with elevation and other factors of the seed-trees' environment; (2) to discover which individual seed trees in each strain, or in each elevational belt, have the inherent ability to produce the most rapidly growing offspring, as determined by measurement of the height, diameter, and branching of the progenies.

The experiment was limited to 729 seed selections or strains, 696 ponderosa pine (*Pinus ponderosa*) and 33 Jeffrey pine (*P. jeffreyi*), gathered from 17 consecutive 500-foot altitudinal zones in what was known as the El Dorado transect, an area in El Dorado County, extending less than 100 miles across the main range of the Sierra Nevada and about 50 miles parallel to the range. Some of the seeds were obtained from the same trees but in different years. In many cases two or more of the seed trees were growing in the same field plot. It was desired to design a nursery in such a way that measurements on progenies would be available free from the influence of all heterogeneity in growing conditions and with an estimate of error adequate for testing the significance of differences between particular progenies.

THEORETICAL BASIS AND DESCRIPTION OF THE DESIGN

The design best adapted to the testing of an unusually large number of varieties is termed the "three-dimensional quasi-factorial" with three groups of sets ("pseudo-factorial" in earlier publications), or more simply, the cubic lattice design (6). The initial requirement is that the number of varieties tested be a perfect cube. In this experiment 729, or 9³, individual seed selections were made. Nine plots or varieties were grouped together to make up a block. It follows, then, that 81 blocks are needed for one complete replication. It was believed that this block size was sufficiently small to eliminate unavoidable heterogeneity therein. Fundamentally this process of arranging fewer than the total number of treatments (individual seed selections) in a block, in other words more than one block to a replication, results in confounding (10). Some of the information on treatment or interaction effects is sacrificed by being entangled with fertility differences between blocks in order that the precision may be increased through a reduction of the standard error due to elimination of block

⁴ A part of the California forest and Range Experiment Station.

differences. Where the heterogeneity of the medium of the experiment is pronounced, the net gain will more than balance the loss in efficiency, as will be demonstrated in this experiment. Here there will be a partial confounding of main effects with block effects.

To overcome this complexity, all the plots in a replication were grouped in the 81 blocks in 3 different ways. The chief requirements for these three were that each individual seed selection be planted not more than once in the same block with any other particular seed selection and that the blocks of each group should cut across those of the other groups. This will be demonstrated in greater detail a little later. Comparisons between the nine progenies within a block may be made directly, but the information on the difference between progenies not occurring in the same block will be entangled with block differences and must be arrived at indirectly through cross-block comparisons. Likewise, the direct information for progenies in the same block will be enhanced by similar cross-block sources. The information available on the differences between pairs of progenies will not be equally precise for all possible comparisons. Instead there will be 3 degrees of precision, hence 3 standard errors, corresponding to the relative position of the seedlings.

The 3 groups, which in the subsequent discussion will be called the X, Y, and Z groups, were each replicated 3 times, making altogether 9 replications, or a total of 6,561 plots in 729 blocks. The only difference between the 3 replications of a group was the independent randomizing of the plots within each block. This design, as will be shown, yields a proper correction factor for each progeny which, when applied to its average, eliminates differences due to block effects.

So extensive an experiment requires some systematic numbering scheme. The method adapted from Yates (7) was to designate each variety (seed selection) by a three-digit number as *uvw*, in which *u*, *v*, and *w* always remain in the order *uvw* but each may take 9 different values. There will be $9^3=729$ different combinations of the 9 numbers, or sufficient for the total number of strains selected. This system is illustrated in figure 1, where a cube of dimension 9 is divided into 729 sections and numbered accordingly. Beginning at the upper front edge and reading to the right, *u* takes on the values 1 to 9 while *v* and *w* remain constant, as 111, 211, 311 911; reading down from the same starting point *w* varies from 1 to 9 while *u* and *v* are constant, as 111, 112, 113 119; finally reading in the third direction, as the *v* arrow points, *u* and *w* are constant and *v* changes from 1 to 9, as 111, 121, 131 191. Each individual seed selection was given one of these 729 numbers which it carried throughout the experiment. No restrictions were placed on the order in which they were made. However, it was desired for technical reasons to have the plots in one replication appear as nearly as possible, without destroying the validity of the test, in the order of the elevation of the seed source, and so the numbers were assigned after the groupings, described in the next paragraph, were effected, and before the plots within the blocks had been randomized.

Referring again to figure 1, the divisions of plots into sets may be readily accomplished for the three groupings mentioned above, heeding only the restrictions that no two strains appear together in the same block in more than one of the groups, and that the sets of each

division be so arranged that they cut across those of all the other divisions. Cutting the cube by two sets of parallel planes through the intersections—the first set parallel to the right-front face and the second parallel to the upper face—yields 81 blocks, each nine plots (small cubes) long. The numbers 111, 211, 311, ..., 911, form a set, another is 112, 212, 312, ..., 912, and so until 81 such blocks are designated. It will be noted that in each of these sets or blocks

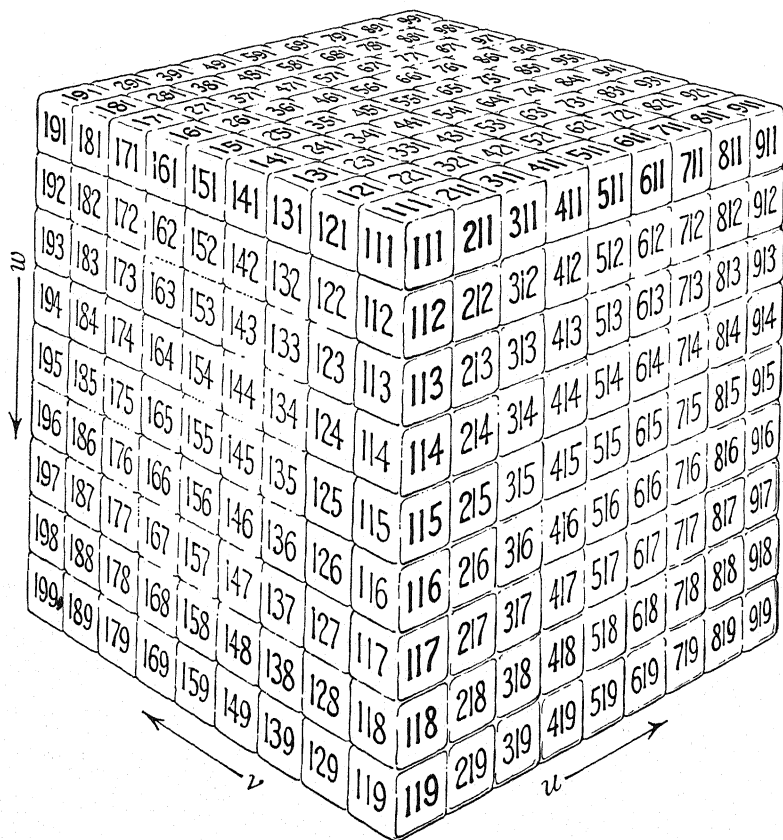


FIGURE 1.—Cube to illustrate the method for determining the sets in the X , Y , and Z groups for the three-dimensional lattice progeny test nursery.

vw numbers are constant and u varies from 1 to 9. This group of 81 sets or blocks was designated by the letter X , and each block number is formed by the combination of its constant vw and small x , e. g., 11 x , 12 x , etc. Group X is given in detail in the first vertical section of table 1.

Passing planes through the cube parallel to the left front face and then horizontally cuts across the sets in the first group making certain that no two plots which appeared together in the X group are now together. The new sets form the Y group. In these u and w are constant and v varies from 1 to 9. One such block is 111, 121, 131, ..., 191; another 911, 921, 931, ..., 991. Consistent with

the first group these would be blocks 11 y and 91 y . The second vertical section of table 1 shows the sets of the Y group.

TABLE 1.—*Numbering system for progenies in the 1937 progeny test nursery, showing block assignments of plots for groups X, Y, and Z*

Block No.	Group X (vw)					Block No.	Group Y ($u-w$)					Block No.	Group Z (uv)				
11 x	111	211	311	---	911	11 y	111	121	131	---	191	11 z	111	112	113	---	119
12 x	112	212	312	---	912	12 y	112	122	132	---	192	12 z	121	122	123	---	129
13 x	113	213	313	---	913	13 y	113	123	133	---	193	13 z	131	132	133	---	139
.
19 x	119	219	319	---	919	19 y	119	129	139	---	199	19 z	191	192	193	---	199
21 x	121	221	321	---	921	21 y	211	221	231	---	291	21 z	211	212	213	---	219
22 x	122	222	322	---	922	22 y	212	222	232	---	292	22 z	221	222	223	---	229
23 x	123	223	323	---	923	23 y	213	223	233	---	293	23 z	231	232	233	---	239
.
29 x	129	229	329	---	929	29 y	219	229	239	---	299	29 z	291	292	293	---	299
31 x	131	231	331	---	931	31 y	311	321	331	---	391	31 z	311	312	313	---	319
32 x	132	232	332	---	932	32 y	312	322	332	---	392	32 z	321	322	323	---	329
33 x	133	233	333	---	933	33 y	313	323	333	---	393	33 z	331	332	333	---	339
.
39 x	139	239	339	---	939	39 y	319	329	339	---	399	39 z	391	392	393	---	399
.
91 x	191	291	391	---	991	91 y	911	921	931	---	991	91 z	911	912	913	---	919
92 x	192	292	392	---	992	92 y	912	922	932	---	992	92 z	921	922	923	---	929
93 x	193	293	393	---	993	93 y	913	923	933	---	993	93 z	931	932	933	---	939
.
99 x	199	299	399	---	999	99 y	919	929	939	---	999	99 z	991	992	993	---	999

The third and last or Z group of sets, which cuts across each of the other two groups, is formed from the cube by passing sets of planes in both vertical directions. The blocks will stand vertically in the figure. Block 11 z will be 111, 112, 113 ----- 119 while block 91 z is made up of plots 911, 912, 913 ----- 919, as in the last vertical section of table 1.

Groups X , Y , and Z were each replicated three times in the nursery, making nine complete replications for each progeny.

DESCRIPTION OF NURSERY BEDS

The most desirable set-up, both from the standpoint of the technical phases and of the field work, was to use beds 4.5 feet by 48 feet, running north and south. Each bed was divided into 288 plots 1½ feet long and 6 inches wide running across the bed, making 3 plots to the width (north, center, and south) and 96 to the length. Nine plots at each end were kept as guard plots, leaving 270 test plots in each bed, which would yield 10 blocks of 9 plots each in each of the north, center, and south positions (figs. 2 and 3A). Each test plot held a row of 6 spots spaced 3 inches apart and planted to 6 seeds each; later these were to be thinned to one seedling per spot. Thus a replication of the 729 seed selections, each represented by 1 plot, required 2.7 seed-beds; and the entire set-up of 9 replications, 24.3 beds.

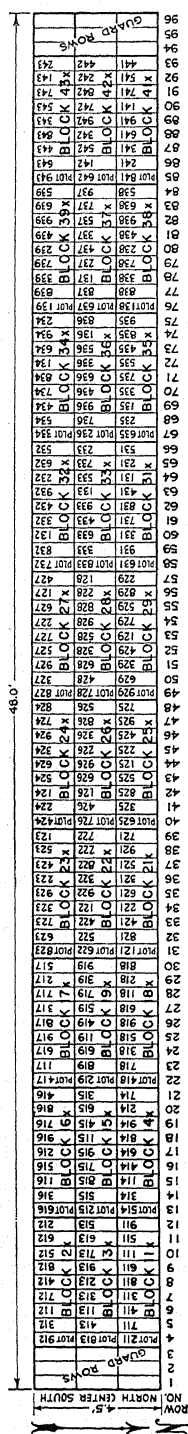


FIGURE 2.—Illustration of arrangement of plots and blocks in a typical nursery bed (bed No. 1).

For the first replication the strains were listed so that certain ones (usually in order of elevation) would appear together in the same block and particular blocks would come in consecutive order, but every precaution was taken to insure that the plot positions within these blocks, as well as those in all the other replications, be determined in a random manner. To equalize any effect due to longitudinal position in the seedbeds each particular block, of which there were three replications, appeared once in each of the three positions, north, center, and south. It was not essential that every plot in the nursery be subject to exactly the same treatment simultaneously—that is, that they be sown, watered, etc., on the same day, or receive the same amount of water—but only that precaution be taken that those within the same block be treated closely alike. To insure a completely random experiment it was decided to randomize the blocks within each of the longitudinal positions with the exception of replication X, in which the blocks occurred in consecutive order. Tippett's Random Sampling Numbers (4) was used for this work.

For recording the field data with efficiency and rapidity in a design as complicated as this, forms must be devised to fit particular measurement, the total for the plot, and the total for the block. In the example here described, the germination count data, days from time of planting until germination, were recorded on the original sowing charts of which figure 2 is a sample.

OUTLINE OF PROCEDURE FOR CORRECTING MEASUREMENTS⁵

As previously stated, the chief purposes of this design were to eliminate differences in progenies which might be due to soil or other treatment heterogeneity under which the individual progenies have been growing and to arrive at a valid estimate of error for making individual

⁵ Very recently Yates has done considerable additional work on the development of methods for the recovery of the interblock information. Since this paper was presented for publication, he has very kindly made available to the authors his results (in manuscript form) on the three-dimensional lattice. By a somewhat different computational procedure from that outlined here, it is possible to determine an estimate of the interblock variance, freed from varietal effects. Adjustments to the varietal means where the interblock and intrablock comparisons are correctly weighted may then be made. While the amount of computation required for this analysis is a little more than that described here, it yields a larger efficiency factor which is also always greater than that for the ordinary randomized blocks, except for the limiting case when there is no reduction of variance due to the use of smaller blocks. When interblock information is ignored, the efficiency factor is not always greater.

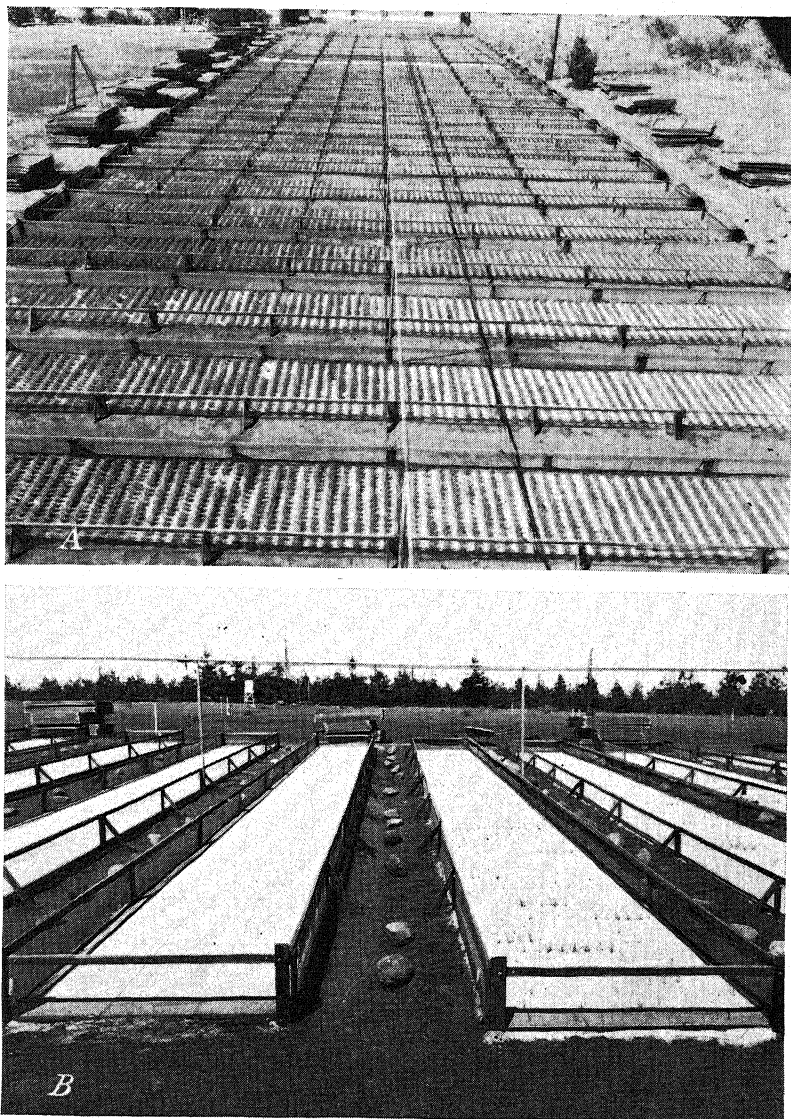


FIGURE 3.—*A*, General view of the entire progeny test experiment. *B*, Bed 16, right, was watered immediately after sowing; bed 17, left, was watered several days later—an example of an unavoidable difference that may occur in treatment and illustrating the need for a design that will eliminate variations due to such causes.

comparisons. The analysis of variance takes the following general form:

Degrees of freedom	Sum of squares	Mean square	F
Incomplete blocks ($3np^2 - 1 = 728$)			
Progenies ($p^3 - 1 = 728$)			
Error ($3np^3 - 3np^2 - p^3 + 1 = 5,104$)			
Total ($3np^3 - 1 = 6,560$)			

Where $p=9$, the number of plots to the block
 $p^2=81$, the number of blocks in each replication
 $p^3=729$, the total number of progenies or strains
 $n=3$, the number of replications of each of the three groups
 $3n=r=9$, the total number of replications
 $3np^2=729$, the total number of blocks in the nursery
 $3np^3=N=6561$, the total number of plots in the nursery

The usual procedure may be followed in computing the sum of squares corresponding to the 728 degrees of freedom for the blocks and the 6,560 making up the total. As has been pointed out earlier, the block differences have been confounded with progenies (varieties) and hence there will be no valid mean square for blocks.

The first step in determining the sum of squares due to progeny differences is to apply a correction factor to the gross average for each variety which will eliminate the differences due to soil and treatment heterogeneity, leaving a value which is truly representative of the particular strain insofar as its characteristics were embodied in the seeds sown. A cubic lattice with dimensions p may be likened to a factorial experiment (7, p. 432) involving three factors each with p values. The main effects of each of these factors would be confounded in two of the three groups or replications and the p^3-1 degrees of freedom (here strains) for treatment would be as follows:

First factor (A)	$p-1$
Second factor (B)	$p-1$
Third factor (C)	$p-1$
Interactions:	
First order (AB, AC, BC)	$3(p-1)(p-1)$
Second order (ABC)	$(p-1)(p-1)(p-1)$
Total	p^3-1

The precision of the estimate of the main effects would be just one-third that of an unconfounded experiment with the same error variance per plot; that for the first order interactions would be two-thirds, while the second order would be entirely free of confounding.

In this progeny test the interest lies in the differences between single progenies rather than main effects and interactions. Estimates of the yield for each variety or strain may be expressed in terms of the gross mean yield, the main effects, and the interactions, the latter two being equivalent to removing differences due to soil or treatment heterogeneity. For factorial experiments involving two or more factors (9, pp. 12-13) the yield of any treatment combination is equal to the mean yield and the sum of plus or minus *one-half* of all the main

TABLE 2.—*Computation of corrected progeny means; First of series of nine; w=1*

SECTION 1: $X_{u,v,w}$												
By groups												
u	v									w		
	1	2	3	4	5	6	7	8	9	1	2	3
1	82	62	72	89	65	76	92	70	66	674	$X_{u,v,w}$	
2	63	90	64	83	98	59	89	86	71	711		
3	64	60	74	95	82	104	78	67	103	730		
4	80	73	101	71	80	73	67	73	73	705		
5	85	74	83	60	93	86	79	98	70	693		
6	67	75	71	88	60	66	79	83	97	69		
7	77	96	111	70	98	116	83	76	70	719		
8	72	91	66	93	72	80	99	76	70	719		
9	89	92	94	95	77	67	89	84	102	789		
$X_{u,v,w}$	679	718	753	742	730	757	766	694	710	6549	$X_{u,v,w}$	
SECTION 2: $Y_{u,v,w}$												
u	v									w		
	1	2	3	4	5	6	7	8	9	1	2	3
1	77	64	66	86	62	75	104	77	74	685	$Y_{u,v,w}$	
2	56	59	63	80	89	74	77	63	79	688		
3	51	59	69	81	74	77	63	79	73	626		
4	76	78	88	66	70	81	74	72	111	716		
5	77	75	81	60	92	77	66	67	75	670		
6	59	65	87	76	82	82	107	72	96	706		
7	67	78	91	63	88	100	73	83	66	709		
8	84	99	64	90	73	85	102	74	84	755		
9	67	82	80	83	63	62	86	73	90	686		
$Y_{u,v,w}$	614	689	689	685	673	703	763	662	763	6241	$Y_{u,v,w}$	
SECTION 3: $Z_{u,v,w}$												
u	v									w		
	1	2	3	4	5	6	7	8	9	1	2	3
1	79	67	72	80	66	68	86	80	63	661	$Z_{u,v,w}$	
2	69	99	61	79	80	59	90	68	67	633		
3	57	49	73	67	82	87	65	86	67	672		
4	76	80	92	58	92	87	72	81	102	740		
5	79	74	84	66	86	79	62	65	68	663		
6	64	67	97	53	61	91	108	66	89	726		
7	63	83	107	58	95	86	69	80	67	708		
8	79	92	71	98	79	84	86	66	66	739		
9	71	83	79	98	62	60	78	67	87	673		
$Z_{u,v,w}$	627	694	736	675	703	701	714	679	676	6215	$Z_{u,v,w}$	

SECTION 4: $T_{u,v,w}$												
For all groups												
u	v									w		
	1	2	3	4	5	6	7	8	9	1	2	3
1	238	193	210	255	193	219	282	267	203	2,020	$T_{u,v,w}$	
2	188	287	183	242	267	182	267	203	247	2,071		
3	172	168	216	243	238	268	196	253	207	1,961		
4	232	231	281	105	241	258	221	220	316	2,195		
5	241	223	281	102	271	242	201	204	216	2,038		
6	190	203	272	239	189	252	313	208	259	2,125		
7	207	257	309	191	281	302	225	280	202	2,234		
8	235	282	201	281	224	249	285	236	220	2,213		
9	227	257	253	264	202	189	253	224	270	2,148		
$T_{u,v,w}$	1,930	2,101	2,178	2,102	2,106	2,161	2,243	2,035	2,149	19,005	$T_{u,v,w}$	

SECTION 5: $T_{u,v,w}/n$												
u	v									w		
	1	2	3	4	5	6	7	8	9	1	2	3
1	26.444	21.444	23.333	28.333	21.444	24.333	31.333	25.222	22.556	-0.124	$C_{u,v,w}$	
2	20.889	31.889	20.889	26.889	29.667	20.222	29.667	22.556	27.444	-333		
3	19.111	18.667	24.000	27.000	26.444	29.778	21.778	23.000	23.000	-1.237		
4	26.778	24.778	27.556	21.333	26.778	28.667	24.556	24.444	35.111	-527		
5	26.778	24.778	27.556	21.333	26.778	28.667	24.556	24.444	35.111	-527		
6	23.000	28.556	30.222	26.556	21.000	28.000	34.778	23.111	28.778	-0.653		
7	26.111	31.333	22.333	31.222	31.222	33.556	25.000	28.889	22.444	-1.664		
8	26.111	31.333	22.333	31.222	31.222	33.556	25.000	28.889	22.444	-1.664		
9	26.222	28.556	28.111	29.333	22.444	21.000	28.111	24.889	31.000	-1.680		
$C_{u,v,w}$.296	-.037	.438	.043	.290	.074	-.531	.056	-1.111			

SECTION 6: CALCULATED CORRECTED PROGENY MEANS, $t_{u,v,w}$												
u	v									w		
	1	2	3	4	5	6	7	8	9	1	2	3
1	27.440	21.760	23.734	30.771	21.982	25.094	31.020	25.555	22.521	25.542	$t_{u,v,w}$	
2	20.577	32.324	21.606	27.797	31.484	29.084	29.058	22.915	28.444	26.145		
3	19.265	19.648	23.924	27.331	25.944	29.371	29.870	27.152	21.376	23.876		
4	26.531	25.883	32.109	23.566	29.569	25.037	24.868	33.166	25.604	27.604		
5	27.390	24.149	26.913	21.178	30.235	27.069	22.630	22.913	23.587	25.118		
6	22.073	31.700	27.355	30.727	28.085	33.795	24.589	28.505	26.872	26.872		
7	23.159	26.931	32.923	20.836	30.727	32.882	33.319	29.028	19.572	26.597		
8	26.998	32.892	22.463	32.483	29.109	28.424	33.689	27.372	27.880	28.361		
9	25.180	28.100	27.468	32.425	21.706	19.662	26.791	24.868	29.145	25.710		
$t_{u,v,w}$	24.290	26.163	26.976	26.638	26.303	26.784	27.357	25.472	25.933			

$\Sigma(X_{u,v,w} t_{u,v,w}) = 171, 768.205.$

$\Sigma(Y_{u,v,w} t_{u,v,w}) = 103, 827.666.$

$\Sigma(Z_{u,v,w} T_{u,v,w}) = 508, 939.949.$

effects and interactions. Analogous to this, the formula (7, p. 436) for the estimate of an individual strain, t_{uvw} , is

$$t_{uvw} = m_{uvw} + \frac{1}{2}(\bar{m}_{..vw} + \bar{m}_{u..w} + \bar{m}_{uv..}) - \frac{1}{2}(\bar{m}_{u..} + \bar{m}_{..v} + \bar{m}_{..w}) \\ + \frac{1}{2}(\bar{X}_{u..} + \bar{Y}_{..v} + \bar{Z}_{..w}) - \frac{1}{2}(\bar{X}_{..vw} + \bar{Y}_{u..w} + \bar{Z}_{uv..}) \text{-----} \quad (1)$$

Where m_{uvw} is the mean yield of the nine replications of a strain, the first and the third quantities within parentheses are considered main effects and the other two interactions. This formula has been developed in a detailed manner, but the procedure has not been included here since it involves considerable algebra inappropriate to the purposes of this article.

For a better understanding of the terms making up the above formula a brief explanation of the mathematical notation is needed. Subtotals of all the plots (3 of these) in each of the three groups which are numbered exactly alike are designated X_{uvw} , Y_{uvw} , and Z_{uvw} , respectively, while the total of the three groups (9 of these) is called T_{uvw} . In table 2 the first item in section 1, 82, is the sum of the three plots in the X group with the number 111; in section 2, 77 is the sum of the three plots in the Y group with the number 111, and finally in section 3, 79 is the sum of the three 111's in the Z group; and the total of all these is 238 in section 4. A dot appearing in place of the u , v , or w in a subscript indicates a summation of all plots whose numbers in the same position as the dot range from 1 to 9; i. e., $X_{..vw}$ is the summation of all plots with the same vw but with u ranging from 1 to 9, and $X_{u..w}$ that of all plots with w constant but all values of u and v . Thus 674 is the sum of the 27 progeny measurements in group X whose numbers end in 11; and 6,549 is the sum of the 243 progeny values in group X with $w=1$, u and v taking on all values from 1 to 9. This notation is extended for all terms with uvw subscripts and is quite adequate for all summations. The corrected progeny mean is denoted t_{uvw} while $t_{..vw}$, $t_{u..w}$, and $t_{uv..}$ are the average progeny means for the nine progenies having constant vw , uw , and uv designations. For ease of computation it has been found desirable to combine certain of the correction terms into three symmetrical parts, designated C_{uvw} , $C_{u..w}$, and $C_{uv..}$. Their composition and consistency will appear from the discussion to follow.

It is now possible to show the application of formula (1) to the data at hand, as follows:

- $m_{uvw} = \frac{T_{uvw}}{3n}$ (section 5 of the first nine computational tables of which table 2 is a sample);
- $\bar{m}_{..vw}$, $\bar{m}_{u..w}$, and $\bar{m}_{uv..}$ = the average of $3np$ (or 81) plots making up $T_{..vw}$, $T_{u..w}$, and $T_{uv..}$, respectively (section 4 of the series of 10 tables);
- $\bar{m}_{u..}$, $\bar{m}_{..v}$, and $\bar{m}_{..w}$ = the average of $3np^2$ (or 729) plots in $T_{u..}$, $T_{..v}$, and $T_{..w}$, respectively (section 4 of the series of 10 tables);
- $\bar{X}_{u..}$, $\bar{Y}_{..v}$, and $\bar{Z}_{..w}$ = the average of np^2 (or 243) plots making up $X_{u..}$, $Y_{..v}$, and $Z_{..w}$, respectively (sections 1 and 2 of the last table of series [table 3], and section 3 of the first 9 [table 2]);

TABLE 3.—*Computation of corrected progeny means: Summary for all values of w*

SECTION 1: $X_{u,v}$											
By groups											
$u \backslash v$		1	2	3	4	5	6	7	8	9	
1	1	727	672	719	785	662	712	724	752	712	6,465 $X_{u,v}$
1	2	719	784	761	666	773	824	758	706	702	6,697
1	3	719	776	674	662	723	824	758	726	743	6,702
1	4	816	822	875	710	789	731	751	808	7,154	
1	5	800	832	789	807	849	743	863	733	782	7,094
1	6	816	807	839	763	784	799	785	805	796	7,094
1	7	817	752	766	748	782	841	749	757	800	7,012
1	8	759	766	718	817	790	788	793	783	764	6,988
1	9	759	795	714	817	693	766	824	730	804	6,877
$X_{u,v}$											
6	6	974	7,006	6,896	6,716	6,836	6,027	6,885	6,753	7,001	62,087 = $X_{u,v}$
SECTION 2: $Y_{u,v}$											
1	1	671	660	676	750	671	712	715	697	706	6,258 $Y_{u,v}$
1	2	737	753	715	675	735	759	745	677	762	6,558
1	3	752	702	650	602	706	733	687	686	668	6,186
1	4	745	725	748	679	699	740	708	714	726	6,484
1	5	737	806	724	715	780	684	712	697	746	6,601
1	6	705	795	753	703	714	778	702	760	776	6,686
1	7	737	697	747	679	749	790	744	740	738	6,621
1	8	730	708	692	762	722	792	747	741	735	6,629
1	9	723	734	714	679	657	716	748	660	759	6,390
$Y_{u,v}$											
6	6	537	6,580	6,419	6,244	6,433	6,704	6,508	6,372	6,616	58,413 = $Y_{u,v}$
SECTION 3: $Z_{u,v}$											
1	1	680	671	726	626	678	684	737	740	666	6,214 $Z_{u,v}$
1	2	798	753	724	636	645	705	781	698	617	6,347
1	3	743	606	639	571	720	755	698	736	701	6,169
1	4	740	748	680	741	711	665	708	684	638	6,384
1	5	722	848	811	771	801	674	647	679	693	6,646
1	6	730	744	743	730	732	821	791	711	708	6,770
1	7	701	708	807	676	736	779	750	655	831	6,703
1	8	670	670	718	785	735	785	621	739	691	6,547
1	9	680	712	718	722	659	757	759	614	700	6,381
$Z_{u,v}$											
6	6	562	6,538	6,719	6,109	6,029	6,489	6,671	6,449	6,411	58,101 = $Z_{u,v}$

SECTION 4: $T_{u,v}$											
For all groups											
$u \backslash v$		1	2	3	4	5	6	7	8	9	
1	1	2,078	2,009	2,121	2,161	2,011	2,108	2,176	2,180	2,084	18,937 $T_{u,v}$
1	2	2,254	2,230	2,200	2,177	2,133	2,192	2,260	2,201	2,171	19,602
1	3	2,311	2,034	1,963	1,855	1,449	2,032	2,143	2,071	2,119	19,057
1	4	2,287	2,307	2,391	2,353	2,430	2,277	2,151	2,173	2,218	20,022
1	5	2,239	2,436	2,324	2,263	2,390	2,108	2,122	2,109	2,221	20,345
1	6	2,211	2,346	2,355	2,303	2,390	2,308	2,243	2,276	2,340	20,550
1	7	2,253	2,217	2,320	2,109	2,267	2,411	2,243	2,152	2,369	20,336
1	8	2,246	2,144	2,263	2,348	2,247	2,365	2,161	2,273	2,190	20,184
1	9	2,162	2,241	2,180	2,159	2,009	2,239	2,331	2,004	2,323	19,648
$T_{u,v}$											
20,063	20,124	20,027	119,022	19,758	20,402	19,842	19,395	20,028	20,028	178,661 $T_{u,v}$	
SECTION 5: CALCULATION OF CORRECTION FACTOR, $C_{u,v}$											
1	1	824	477	687	2,519	372	811	342	401	1,200	
1	2	-275	805	612	1,198	1,890	941	255	636	2,644	
1	3	1,095	2,235	723	1,525	447	1,756	860	222	1,664	
1	4	1,984	780	976	2,338	-028	1,355	1,359	896	1,663	
1	5	1,163	235	-234	649	681	953	1,675	1,037	1,545	
1	6	1,719	1,317	1,093	809	728	964	-389	1,475	-891	
1	7	1,527	076	-184	1,235	879	916	514	1,747	-067	
1	8	1,435	1,440	-524	1,062	774	527	2,397	1,938	1,381	
1	9	1,342	1,261	599	729	712	268	891	1,565	954	
$C_{u,v}$											
SECTION 6: CALCULATED CORRECTED PROGENY MEANS, $I_{u,v}$											
1	1	26,044	24,799	26,056	28,653	24,770	26,290	26,680	26,873	26,308	
1	2	27,258	28,736	27,697	25,200	28,151	27,597	28,190	25,791	28,965	
1	3	28,556	26,867	24,105	22,998	25,918	28,118	26,155	25,552	25,541	
1	4	27,832	27,828	29,326	24,996	26,521	27,968	26,653	26,924	27,502	
1	5	28,021	29,868	27,644	26,655	25,749	26,750	26,750	26,424	27,747	
1	6	27,168	29,386	28,281	26,962	27,412	28,710	26,044	28,607	28,746	
1	7	28,554	26,587	27,863	26,863	28,059	28,745	27,301	27,384	28,151	
1	8	27,416	27,115	26,021	29,155	27,773	28,866	28,237	28,134	27,474	
1	9	27,023	27,870	26,720	26,262	24,509	26,788	28,566	25,205	28,416	
$I_{u,v}$											

$\Sigma(Z_{u,v}/f_{u,v}) = 1,587,292.080$

SECTION 4: $T_{u,v}$

$u \backslash v$		For all groups									
		1	2	3	4	5	6	7	8	9	
1	1	2,078	2,009	2,121	2,161	2,011	2,108	2,176	2,189	2,084	18,937
1	2	2,254	2,290	2,200	1,977	2,153	2,102	2,234	2,071	2,171	19,602
1	3	2,311	2,084	1,963	1,855	2,149	2,312	2,143	2,148	2,112	19,057
1	4	2,259	2,307	2,391	2,324	2,262	2,277	2,192	2,109	2,218	20,022
1	5	2,287	2,486	2,324	2,293	2,300	2,308	2,276	2,276	2,221	20,345
1	6	2,211	2,346	2,335	2,196	2,320	2,398	2,218	2,218	2,340	20,550
1	7	2,211	2,346	2,320	2,103	2,307	2,410	2,243	2,152	2,369	20,336
1	8	2,256	2,444	2,320	2,345	2,247	2,365	2,161	2,273	2,190	20,184
1	9	2,162	2,241	2,180	2,139	2,009	2,239	2,331	2,004	2,323	19,648
$T_{u,v}$		20,063	20,124	20,027	119,022	19,758	20,402	19,842	19,305	20,028	=178,661 $T_{u,v}$

SECTION 5: CALCULATION OF CORRECTION FACTOR, $C_{u,v}$.

SECTION 5: CALCULATION OF CORRECTION FACTOR, $C_{u,v}$

u	v	1	2	3	4	5	6	7	8	9
1	1	.824	.477	.087	2,519	.372	.811	.342	.401	1,200
1	2	-.275	.805	.612	1,198	1,800	.941	.235	.636	.264
1	3	1,095	2,255	.723	1,525	.447	.756	.860	.222	.724
1	4	.984	.780	.976	2,383	-.023	1,355	1,339	.895	1,693
1	5	1,163	.255	-.234	.649	.681	.953	1,075	1,037	1,545
1	6	.719	1,317	1,093	.809	.724	.004	.899	1,475	.891
1	7	1,527	.076	-.184	1,235	.879	.916	.514	1,747	-.087
1	8	1,435	1,440	-.524	1,062	.774	.527	.397	.868	1,391
1	9	1,342	1,261	.699	.729	.712	.208	.891	1,553	.384

SECTION 6: CALCULATED CORRECTED PROGENY MEANS, $t_{u,v}$

u	v	1	2	3	4	5	6	7	8	9
1	1	26,044	24,799	26,056	28,653	24,770	26,290	26,680	26,873	26,308
1	2	27,258	28,736	27,097	25,200	28,151	27,597	26,190	25,791	28,965
1	3	28,556	26,867	24,105	22,998	26,918	28,118	26,155	25,652	25,541
1	4	27,832	26,867	27,644	24,946	26,521	27,968	26,653	26,216	27,502
1	5	28,021	29,868	27,644	27,815	29,655	25,749	26,750	25,924	27,747
1	6	27,108	29,386	28,281	26,902	27,412	28,710	26,044	28,077	28,746
1	7	28,554	26,387	27,863	26,274	28,059	29,745	27,301	27,384	28,151
1	8	27,416	27,110	26,021	20,155	27,773	28,866	28,237	28,134	27,494
1	9	27,023	27,875	26,720	26,262	24,509	26,788	25,566	25,205	28,416

And, finally,

$\bar{X}_{v.w}$, $\bar{Y}_{u.w}$, and $\bar{Z}_{u.v}$ = the average of np (or 27) plots in $X_{v.w}$, $Y_{u.w}$, and $Z_{u.v}$, respectively, sections 1 and 2 of the first 9 tables [table 2] and, section 3 of the last of series [table 3].

For computational work a slightly different form has been found most suitable. Substituting the foregoing in formula (1) gives—

$$t_{uvw} = \frac{T_{uvw}}{3n} + \frac{1}{2} \left[\frac{T_{v.w}}{3np} + \frac{T_{u.v}}{3np} + \frac{T_{u.w}}{3np} \right] - \frac{1}{2} \left[\frac{T_{u..}}{3np^2} + \frac{T_{v..}}{3np^2} + \frac{T_{..w}}{3np^2} \right] \\ + \frac{1}{2} \left[\frac{X_{u..}}{np^2} + \frac{Y_{v..}}{np^2} + \frac{Z_{..w}}{np^2} \right] - \frac{1}{2} \left[\frac{X_{v.w}}{np} + \frac{Y_{u.w}}{np} + \frac{Z_{u.v}}{np} \right]$$

which may be reduced to

$$t_{uvw} = \frac{T_{uvw}}{3n} + \frac{1}{6np^2} \left[pT_{v.w} - 3pX_{v.w} - T_{v..} + 3Y_{v..} \right] + \frac{1}{6np^2} (pT_{u.w} \\ - 3pY_{u.w} - T_{..w} - 3Z_{..w}) + \frac{1}{6np^2} [pT_{u.v} - 3pZ_{u.v} - T_{u..} + 3X_{u..}]$$

With the terms containing the brackets represented by $C_{v.w}$, $C_{u.w}$, and $C_{u.v}$, the formula for correcting an individual progeny becomes—

$$t_{uvw} = \frac{T_{uvw}}{3n} + C_{v.w} + C_{u.w} + C_{u.v}. \quad (2)$$

It will be seen later that the computation of the C 's is a very simple matter. $C_{v.w}$ and $C_{u.w}$ appear in the margins of section 5, table 2, $C_{u.v}$ in section 5, table 3. Table 2, section 6, gives the corrected mean germination days of the 81 progenies so numbered that $w=1$. There will be similar sections for w equal to 2, 3, ----- 9.

Using the corrected progeny means it would be possible to find the sum of squares for progenies by the usual procedure. It can be proved, however, that the same results may be arrived at with the formula—
SS (corrected progeny total)

$$= \Sigma(t_{uvw}T_{uvw}) - [\Sigma(X_{v.w}t_{v.w}) + \Sigma(Y_{u.w}t_{u.w}) + \Sigma(Z_{u.v}t_{u.v})] \quad (3)$$

and with very much less work.

Had there been no confounding in this experiment, the variance of every comparison between pairs of progenies would have been $\frac{2s^2}{r}$, s being the error value determined from the analysis of variance table and r the number of replications. Because of the confounding, pairs of progenies will be classified in three ways on the basis of their relative block locations, for comparison by means of the variance of the mean difference. The variance for each of these is expressed in the following three formulas—

$$V(t_{211} - t_{111}) = \frac{2s^2}{rp^2}(p^2 + p + 1) \text{ -----} (4)$$

$$V(t_{122} - t_{111}) = \frac{s^2}{rp^2}(2p^2 + 3p + 4) \text{ -----} (5)$$

$$V(t_{222} - t_{111}) = \frac{s^2}{rp^2}(2p^2 + 3p + 6) \text{ -----} (6)$$

depending on whether the progenies differ in one, two, or three of the letters *uvw*.

The mean variance of all comparisons is—

$$V_m = \frac{s^2 (2p^2 + 5p + 11)}{r(p^2 + p + 1)} \text{ ----- (7)}$$

Formulas 4, 5, and 6 for variances of mean difference were derived in the same manner as were the formulas for correcting progeny means, in terms of main effect and interactions (7, pp. 433-437).

The resulting standard errors from extracting the square roots of each of the four variances are the error factors to be used in making individual comparisons of progenies by "Student's" *t* test.

The factor by which each of the above variances differ from $\frac{2s^2}{r}$

is a measure of the increase in variance that results from the division of the varieties into sets when the error variance per plot is unaltered by the resultant reduction in block size. The reciprocal of such factor, called efficiency factor of the arrangement (9, p. 86), is a measure of the inherent strength of the arrangement.

Thus the increases in variance are—

$$\begin{aligned} \frac{2s^2}{rp^2}(p^2 + p + 1) \div \frac{2s^2}{r} &= \frac{p^2 + p + 1}{p^2} \\ \frac{s^2}{rp^2}(2p^2 + 3p + 4) \div \frac{2s^2}{r} &= \frac{2p^2 + 3p + 4}{2p^2} \\ \frac{s^2}{rp^2}(2p^2 + 3p + 6) \div \frac{2s^2}{r} &= \frac{2p^2 + 3p + 6}{2p^2} \end{aligned}$$

and for all comparisons—

$$\frac{s^2}{r} \frac{2p^2 + 5p + 11}{p^2 + p + 1} \div \frac{2s^2}{r} = \frac{2p^2 + 5p + 11}{2(p^2 + p + 1)}$$

With $p=9$, the efficiency factor is in each case then—

$$\frac{p^2}{p^2 + p + 1} = .890,$$

$$\frac{2p^2}{2p^2 + 3p + 4} = .839,$$

$$\frac{2p^2}{2p^2 + 3p + 6} = .831,$$

and for the mean variance of all comparisons—

$$\frac{2(p^2 + p + 1)}{2p^2 + 5p + 11} = .835$$

APPLICATION OF PROCEDURE TO GERMINATION DATA

With the preceding notation and formulas as a basis, the actual computations involved in correcting the progeny means for heterogeneity are very simple. The process can best be carried through in tabular form using a series of 10 computational tables of six sections each, of which table 2 illustrates the first ($w=1$) and table 3 the last—a summary of the other nine tables. For illustrative material, the time of germination was recorded for each of the 6,561 plots. This time was defined as the number of days from planting until the first day when three or more spots (six spots to the plot) had one or more seedlings (six seeds planted to the spot) visible above the ground regardless of whether or not they had been injured by damping-off or otherwise.

The procedure may be demonstrated as follows:

(1) Using a convenient index for finding the location in the nursery of the replications of individual progenies, the first step is to find the sum of the three plot measurements having the same uvw number in each of the three groups and record them in tabular form. For example, in table 2, for totals in the X group, the readings for No. 111 were $31+27+24=82$; in the Y group, the readings for the same progeny were $28+27+22=77$; and in the Z group they were $28+26+25=79$. This process is repeated for each of the other 728 progenies.

(2) The sum of the three totals above, $82+77+79=238$, or T_{111} , the total for the 9 replications of progeny No. 111. This and similar totals for the progenies whose numbers end in 1 are recorded in the fourth section of table 2.

(3) Marginal totals are found for the first four sections yielding $X_{u \cdot w}$, $X_{v \cdot w}$, $X_{\cdot w}$, and these are repeated for Y , Z , and T , as indicated in table 2.

(4) The average $\frac{T_{uvw}}{9}$ is computed for each value of T in the nine tables—a very simple matter. These form the fifth section of each. In table 3, for No. 111, $\frac{238}{9}=26.444$.

(5) For X_{uv} , the items occupying the same position in each of the nine tables are added. The first summation is $82+93+84+70+68+74+97+90+69=727$. Of these, the first value only, 82, may be found in table 2. This result, 727, is the first item in the tenth table, here represented by table 3. This process is repeated for each of the other progenies.

(6) Marginal totals here yield $X_{u \cdot \cdot}$ and $X_{\cdot \cdot}$. The same procedure is followed for Y , Z , and T .

(7) The next important step is the calculation of the correction factors C_{vw} , $C_{u \cdot w}$, and $C_{u \cdot \cdot}$. Substituting in the formulas given above for these:

$$C_{vw} = \frac{1}{6 \times 3 \times 9^2} [9 \times 2020 \text{ (table 2)} - 27 \times 674 \text{ (table 2)} - 18,937 \text{ (table 3)} + 3 \times 6258 \text{ (table 3)}] = -0.124 \text{ (vertical margin of fifth section, table 2).}$$

$$C_{uw} = \frac{1}{6 \times 3 \times 9^2} [9 \times 1930 \text{ (table 2)} - 27 \times 614 \text{ (table 2)} - 19,005 \text{ (table 2)} + 3 \times 6215 \text{ (table 2)}] = 0.296 \text{ (horizontal margin of fifth section, table 2).}$$

$$C_{uv} = \frac{1}{6 \times 3 \times 9^2} [9 \times 2078 \text{ (table 3)} - 27 \times 680 \text{ (table 3)} - 20,063 \text{ (table 3)} + 3 \times 6974 \text{ (table 2)}] = 0.824 \text{ (fifth section, table 3).}$$

Attention is called to the fact that the coefficients of these three formulas are such as to give equal weighting to all items. The sum of all the C 's should equal zero, which is a check on the accuracy of the computations.

(8) The final operation in correcting the individual progenies for heterogeneity is to apply these correction values to the original average in section 5, table 2, using formula 1—

$$26.444 - 0.124 + 0.296 + 0.824 = 27.440$$

which is the corrected number of days from planting until germination for progeny No. 111.

With each of the 729 progeny means corrected, it is a very simple matter to carry through the computations necessary for obtaining the items in the analysis of variance table form above. If d_{111} , d_{211} d_{999} be the original germination time, then summing over all replications—

$$\Sigma d_{uvw} = 178,661, \text{ and, the, correction, factor, becomes } \frac{(178,661)^2}{6561} = 4,865,074.37,$$

$$\Sigma d_{uvw}^2 = 5,122,921.$$

$$\text{Total } SS = 5,122,921 - 4,865,074.37 = 257,846.63.$$

For the variation due to blocks, b ,

$$\Sigma b^2 = 44,601,697$$

$$SS \text{ due to blocks} = \frac{44,601,697}{9} - 4,865,074.37 = 90,669.74$$

The usual procedure for finding the sums of squares for the corrected progeny measurements could have been used, but formula (3) simplifies and shortens the labor to a considerable degree. Substituting therein—

$$SS \text{ due to progenies} = 5,011,580.822 - [1,689,976.220 + 1,592,598.640 + 1,587,292.030] = 141,713.932.$$

These known items, may now be tabulated for the analysis of variance and the error term, 25,462.96, obtained by subtraction, table 4.

TABLE 4.—Analysis of variance of the germination period in days

Variation due to	Degrees of freedom	Sum of squares	Mean square	F
Blocks.....	728	90,669.74
Progenies.....	728	141,713.93	194.66	139.02
Error.....	5,104	25,462.96	4.988824
Total.....	6,560	257,846.63

¹ Highly significant.

The error mean square in table 4 is the squared standard error, that is, $s^2=4.988824$. The comparison of individual progeny means falls into three groups, which may be determined from the progeny number, with distinct standard errors of the difference. These are expressed in formulas (4) to (7). Substituting in these

$$V(t_{211}-t_{111})=\frac{2 \times 4.988824}{729} \times 91 = 1.245495 SE = 1.116$$

$$V(t_{122}-t_{111})=\frac{4.988824}{729} \times 193 = 1.320772 SE = 1.149$$

$$V(t_{222}-t_{111})=\frac{4.988824}{729} \times 195 = 1.334459 SE = 1.155$$

Mean variance of all comparisons

$$V_m = \frac{4.988824}{9} \times \frac{218}{91} = 1.327917 \quad SE_m = 1.152$$

With these standard errors it is now possible to make any individual comparisons of means desired using the well-known t test.

$t = \frac{\text{Mean}_1 - \text{Mean}_2}{SE}$ and referring to the t table (1, *p. 166*) for the probability that the difference might be due to random sampling. This t is, of course, the one first established by "Student" in 1908. An adequate treatment of it is given by Fisher (1). To compare progenies No. 111 and No. 211.

$$t = \frac{21.760 - 27.440}{1.116} = -5.090$$

Entering the t table (1, *p. 166*) at $n=16$ ($n=n_1+n_2$ where n_1+1 and n_2+1 are each equal to 9), the computed value of t , 5.090, is found to be far beyond the range of the table, showing that the probability, P , is extremely small. This justifies the conclusion that the seeds from the tree whose progeny is No. 111 germinate at a slower rate than those from the seed tree of progeny No. 211.

In an earlier paragraph the efficiency factors for this design were computed. For the mean variance of all comparisons, this efficiency factor was found to be 0.835. In other words, 16.5 percent was lost because the ordinary randomized block per replication was not used. The reduction in error variance due to the design will, however, more than compensate for this loss. It is possible to take into account the information accruing from the block comparisons, since this experiment has a sufficient number of replications (nine in all with three for each of the groups X , Y , and Z) to give an adequate estimate of error for interblock as well as the intrablock comparisons. This adds greatly to the attractiveness of the design.

To use the information from the interblock comparisons most accurately, all the blocks forming a complete replication X , Y , or Z should be arranged in a compact unit on the ground, with these three groups randomized for positions with reference to each other in addition to the randomization of blocks within groups and plots within blocks (9, *pp. 30, 31, 86*). Although utilization of interblock compari-

sons has been introduced since the experiment was installed and the pattern outlined was not exactly followed, still it is possible to recover much of the lost information. With the present design, the 728 degrees of freedom ascribed to blocks (these confounded with main effects) may be broken down as follows:

	<i>D/f</i>
Groups-----	2
X grouping:	
Blocks-----	80
Error-----	162
Y grouping:	
Blocks-----	80
Error-----	162
Z grouping:	
Blocks-----	80
Error-----	162
	<hr/> 728

Combining the three terms for error gives 486 degrees of freedom. $\frac{\Sigma(X_{vw}) + \Sigma(Y_{u.w}) + \Sigma(Z_{uv.})}{27}$ minus the correction factor will give the part of the sum of squares for blocks other than error. Subtracting this from the total sum of squares due to blocks leaves the error term attributed to the 486 degrees of freedom.

This computation becomes

$$\frac{48,003,111 + 42,409,381 + 42,040,325}{27} - 4,865,074.37 = 40,585.52.$$

$$90,669.74 \text{ (SS due to blocks)} - 40,585.52 = 50,084.22.$$

This may be set up in the following form:

Item	<i>D/f</i>	<i>SS</i>	<i>MS</i>
Blocks-----	486	50,084.22	103.05
Within-----	6,074	4.988824	
Total-----	6,560		12.25

The mean square for the 6,560 degrees of freedom, 12.25, is found thus:

$$\frac{50,084.22 + (6074 \times 4.988824)}{6560} = 12.25$$

Following Fisher (2, *pp.* 255-258), the expression for precision is $\frac{n+1}{(n+3)s^2}$, where n is the degrees of freedom and s^2 the sampling variance. Hence the ratio of the two sampling variances above, $\frac{12.25}{4.99} = 2.45$, or 245 percent, will measure the recovery of information due to reduction in error variance by the use of this design. This means that the experiment is about $2\frac{1}{2}$ times as precise as it would have been if the ordinary randomized block design had been used.

Earlier, account was taken of the loss of information due to the confounding of main effects with blocks, with the result that the

efficiency of the experiment was assessed at 0.835. The net efficiency from these two sources is then

$$0.835 \times 2.45 = 2.05$$

This net gain of 105 percent makes it evident that the use of the cubic lattice design for this experiment was most worth while.

EXPLANATION AND SUMMARY

An examination of the body of germination data after the correction factors have been applied gives most conclusive evidence of the effectiveness of this type of design for the purpose for which it was evolved, namely, to eliminate differences in yields or measurements due to soil or treatment heterogeneity. Besides the expected soil variations, a known variable factor was introduced in the watering time of the nursery. It is granted that watering will tend to hasten germination. The entire nursery was watered once, beds 1-16 on April 27 and beds 17-25 on May 7. The sowing started on April 20 with bed 1 and continued consecutively until finished on May 5. This means that the greatest number of days between planting and watering for the first set would have been for bed 1, and the least for bed 16; in the second set the greatest number of days for bed 17, the lowest number of days for bed 25 (fig. 3 B).

The number of days from watering to germination were also recorded for each of the 6,561 plots. Using the original average values, time to germinate from planting date minus the time to germinate from watering varied from plot to plot, a range of 3.3 to 5.9.

When the average number of days from watering time to germination for each progeny was corrected, as was done for the planting time, the differences between corrected planting and corrected watering time became practically constant at 4.6 days. This is evidence that this design does eliminate effects of plot differences upon the average values, whether they be initial or some later happening, as in the incident of watering the nursery.

The correction of the 729 progeny means for heterogeneity due to location and the setting up of the procedure for making tests of significance of the differences of these individual progeny means completes the objectives of this article. From the standpoint of the purposes for which this experiment was designed, however, it marks only the initial step in the selection of seed trees for the improvement of the strains of timber trees; it is necessary to learn what conditions affect the growing characteristic of the seedlings. Hence, the next step, which is beyond the scope of this article, would be to subject the corrected data to such standard statistical procedures as seem most applicable.

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ELECTRICAL STIMULATION OF ISOLATED HEART PREPARATIONS FROM PERIPLANETA AMERICANA¹

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INTRODUCTION

In order to study adequately the effects of insecticides upon the various tissues and functional processes of insects, particularly insects which it is desired to control, it is necessary to have more knowledge of the manner in which the tissues and organs of insects function under normal and abnormal conditions. This paper is a report of experiments which yielded further information regarding the functional processes of the insect-cardiac mechanism.

In utilizing the mechanocardiographic method of studying the effects of nicotine and other solutions upon a perfused, isolated heart preparation from the American cockroach (*Periplaneta americana* (L.)) it is necessary to consider the effects of possible contractions by other than cardiac-muscle fibers. In addition to the cardiac-muscle fibers, the cockroach isolated heart preparation previously used (4, 5, 6, 7)² possessed alary muscles (of the heart), dorsal body muscles, and, in the thoracic region, especially of heart preparations from the adult insect, portions of the muscles involved in the somatic wing mechanisms.

It has been shown previously (4) that the systolic rise of curve in the mechanocardiogram taken from the whole isolated heart preparation of *Periplaneta americana* is often immediately preceded by a sudden depression, the presystolic notch. It has been suggested (4), on the basis of certain unpublished evidence, that the presystolic notch might result from an increased intracardiac pressure (hydrostatic) produced by a propagated wave of heartbeat in a cardiac region other than that from which the mechanocardiographic record is being taken. Although under certain conditions the presystolic notch may originate in this manner, the possibility still remains that under other conditions it might originate from a rhythmic presystolic contraction of the alary-muscle fibers of the heart, especially in the segment from which the record is being made. If the presystolic notch originates in accordance with this hypothesis, it should be possible to obtain supportive evidence by artificially stimulating the isolated heart preparation, especially the alary-muscle fibers in the segment in which the heart lever is attached.

The experiments herein reported were performed with this in mind, but also with the general objective of determining how the isolated heart preparation of *Periplaneta americana* responds to single and repeated faradic stimuli and how these responses compare with the better known results of artificial stimulation of the vertebrate heart.

¹ Received for publication December 12, 1938.

² Italic numbers in parentheses refer to Literature Cited, p. 137.

METHODS

As described below, four different types of isolated heart preparations were used in these experiments.

Type A, the entire isolated heart preparation that was utilized in previous investigations (5, 6, 7) was used in some of these experiments. The preparation consisted of the beheaded insect's dorsal body wall (terga), dissected from the rest of the body but retaining the cardiac mechanism. It contained the cardiac tube, the alary-muscle-fiber groups, the external and internal dorsal body muscles, the dorsal diaphragm, the fat bodies, trachea, and other incidental structures associated with the dorsal diaphragm and the terga, and, in the thorax, the severed somatic wing muscles.

Type B, the second preparation, consisted of only the abdominal region of the heart, prepared either by transverse section across preparation A between the third thoracic and the first abdominal segments or by separating by similar cross section the abdomen from the thorax of the living insect and subsequently isolating the abdominal heart preparation by dissection. Whichever procedure was followed, the resulting preparation was the same and contained the entire abdominal but not the thoracic portions of the cardiac mechanism. In this preparation the longitudinal internal and external dorsal body muscles of the abdomen remained intact and retained their normal origins and insertions.

Type C, the third preparation, consisted of a single abdominal segment separated by transverse section with a safety-razor blade from adjacent posterior and anterior segments of either preparation A or preparation B (usually the latter) but cut in such a way that the adjacent margin of the adjacent overlapping segment remained attached to the segment being isolated. This preparation of a single abdominal segment contained a single pair of alary muscle-fiber groups and only that length of cardiac tube that lay between their cardiac terminations. The internal dorsal body muscles were transected, and only the ends attached to the isolated segment remained in the preparation. The external dorsal body muscles were not severed.

Type D, the fourth preparation, was made in the same way as preparation C except that the adjacent margin of the adjacent overlapping segment was dissected away and the connections of the external dorsal body muscles therefore destroyed. This preparation was thus completely separated from both anterior and posterior adjacent segments.

During some of these experiments the isolated heart preparation was kept moist by occasional flooding with approximately 0.11 Lévy's (3) stock saline solution (prepared without the buffers). This saline was composed of 11.78 gm. of sodium chloride, 0.92 gm. of potassium chloride, and 0.66 gm. of calcium chloride per liter of solution. In the other experiments the heart preparation was continuously perfused with a stream of another saline solution that was made to flow over the exposed tissues. The formula for the composition of this saline solution was arrived at in the following way: Mixtures were made containing known proportions of the 0.11 Lévy's solution described above and of a saline (composed of 3.26 gm. of sodium chloride, 7.39 gm. of potassium chloride, 2.54 gm. of calcium chloride, and 1.69 gm. of magnesium chloride per liter of solution)

prepared by Babers on the basis of his analysis (1) of the blood of the mature larva of the southern armyworm (*Prodenia eridania* (Cram.)). These mixtures were used to perfuse the isolated heart preparation of *Periplaneta americana*, and that mixture was chosen which sufficed to maintain the perfused heart preparation in the most steady state with respect to both rate and amplitude of cardiac contractions. The selected mixture was found by calculation to have the composition 10.93 gm. of sodium chloride, 1.57 gm. of potassium chloride, 0.85 gm. of calcium chloride, and 0.17 gm. of magnesium chloride per liter of solution. This solution will be referred to in this paper as the magnesium saline, the 0.11 Lévy's solution will be referred to as Lévy's saline. The perfusion saline was oxygenated by bubbling oxygen through the reservoir bottle before and during the experiment.

The isolated heart preparation was held by pins in a beeswax container so constructed that the perfusion saline flowed over the exposed cardiac mechanism into a surrounding drainage depression from which it flowed into a collecting jar. The drainage depression of the wax container surrounded a central wax elevation or plateau one edge of which was higher than the other. Drainage was facilitated by laying narrow strips of wet lens paper in the drainage depression. The heart preparation used in each experiment was pinned to the wax plateau with the external dorsal surface down and the internal cardiac mechanism (dorsal diaphragm) up. The rate of flow of perfusion fluid over the exposed cardiac tissues was adjusted by means of a stopcock until it was as rapid as possible without mechanically interfering with the hair attachment or the mechanocardiogram. This method of perfusion was somewhat different from that used in previous studies of the rate of insect heartbeat (5, 6, 7).

The apparatus used to stimulate the heart preparation electrically consisted of the well-known student-type inductorium, key, signal magnet, dry cells, and a specially made pair of microelectrodes with tungsten tips. The signal magnet was included in the primary circuit and placed so that its lever would intercept the light passing through the end of the camera slit upon closure of the key of the primary circuit. Thus the shadow of the signal magnet appeared in the mechanocardiograms as lower marginal bands during the times the key of the primary circuit was closed. When single induced shocks were used they were applied to the tissue at times indicated by the beginning and the end of the marginal band. When the inductorium was so wired as to supply tetanizing shocks they were applied to the tissue in rapid succession throughout the duration of the period indicated by the marginal band. The time record, consisting of lines extending completely across the photographic paper, were made by a Lieb watch timer, the lever of which was made to cast a shadow across the entire camera slit at determined time intervals (1 second in these experiments).

The complete mechanocardiographic set-up of apparatus was essentially the same as that previously described (4). The electrocardiographic camera, however, has been remodeled to yield paper speeds ranging from about 0.5 to about 100 mm. per second. In order to adapt intensity of light to paper speed, the beam of light was passed through suitable filters. The records were made upon a roll of bromide photographic paper. Most of the experiments were

performed at room temperature, which was maintained fairly constant and usually at a temperature lying within the range 25° to 30° C.

Unless otherwise stated, the lever contact was with the dorsal diaphragm immediately over the cardiac tube. As the cardiac-muscle fibers contracted, the dorsal diaphragm and lever attachment were lowered and the lever moved so as to produce an upward deflection of the mechano-cardiographic curve and, conversely, when the diaphragm was raised the curve was depressed.

Similarly, upward or downward deflections of the lever shadow on the photographic paper were produced when the diaphragm was lowered or raised by any other cause, as for example by movement of the preparation resulting from contractions of the dorsal body muscles. The position of the electrodes was changed from time to time as indicated in the legends of the illustrations and under the heading Results. The stimuli applied consisted of single induced shocks, induced shocks repeated by opening and closing the key of the primary circuit by hand, and series of induced tetanizing shocks applied at a rate determined by the vibrator of the inductorium. Because of current leakage through the saline from one electrode to another, a rather strong stimulating current was employed.

All the mechanocardiograms obtained in these experiments are to be read from left to right.

RESULTS

EXTRASYSTOLES AND SUMMATION OF CONTRACTIONS

Figure 1, *A*, shows the regularity in rate and amplitude of heart contraction when the completely isolated segment (preparation D) was perfused continuously with the magnesium saline solution. *A* and *B* (preparation C) are parts of records

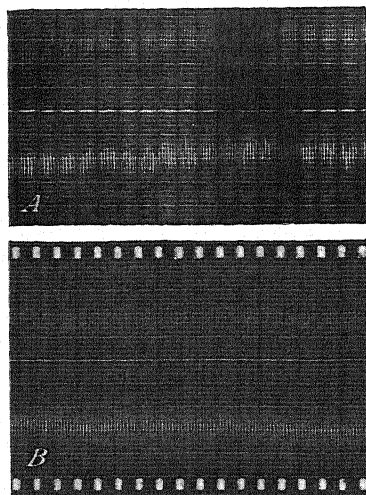


FIGURE 1.—*A*, Mechanocardiogram from a completely isolated fourth abdominal segment (preparation D) perfused with the oxygenated magnesium saline. Room temperature 29° C.; record taken 105 minutes after beginning of perfusion; lever over heart; electrodes in place on distal extremity of alary-muscle group; paper speed about 0.5 mm. per second; time markings in seconds; no electrical stimuli. *B*, Similar record from an incompletely isolated third abdominal segment (preparation C). Room temperature and perfusion as in *A*; preparation 105 minutes old; lever over heart; no electrodes.

taken at low paper speed in different experiments after each of the preparations had been perfused for about 105 minutes.

The results of applying single and repeated faradic stimuli to the completely isolated fourth abdominal segment (preparation D) are shown in figures 2, 3, and 4. Figure 2 (*A*, *a*; *B*, *a*) shows the extrasystoles produced by stimulating with single induced shocks during diastasis (the rest period of the cardiac cycle). These extrasystoles are a little greater than the spontaneous contractions (fig. 2, *A*, *c* and *d*; *B*, *c* and *d*).

In figure 3 the extrasystoles at *A*, *a* and *b*, and at *B*, *a*, were produced by single shocks applied at different times (*A*, *c* and *d* and *B*, *b*)

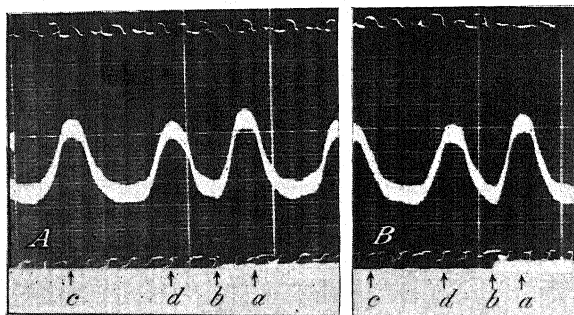


FIGURE 2.—Mechanocardiograms from a completely isolated fourth abdominal segment (preparation D), 145 minutes old. Room temperature 29° C.; perfusion with magnesium saline; lever over heart; electrodes over lateral extremity of alary-muscle group; time markings in seconds; and stimuli single induced shocks. *A*, Extrasystole at *a* produced by stimulus applied during late diastasis at *b*; *c* and *d* are spontaneous contractions. *B*, Extrasystole at *a* produced by stimulus applied during early diastasis at *b*; *c* and *d* are spontaneous contractions.

during diastole (relaxation period). The summation effect in *B* is greater than that in *A*. In figures 2 and 3 the electrodes were at the lateral extremity of an alary-muscle group.

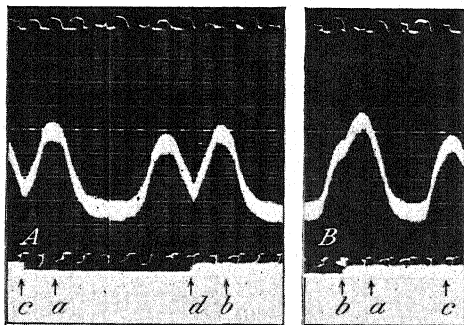


FIGURE 3.—Mechanocardiograms from a fourth abdominal segment (preparation D). Room temperature 29° C.; perfusion with magnesium saline; lever over heart; electrodes over lateral extremity of alary-muscle group; single induced shocks applied. *A*, Extrasystoles at *a* and *b* caused by single shocks applied during late diastole at *c* and *d*, respectively. *B*, Extrasystole at *a* caused by a single shock applied at *b* during the beginning of the diastole of a spontaneous beat; *c* is a spontaneous contraction.

Similarly figure 4 shows the effect of applying single shocks (electrodes medially over an alary-muscle group) at different times during systole (contraction period). Apparent summation effects are produced. In figure 4, *B*, *d*, summation at the height of contraction is very evident. The shock applied at *A*, *c*, may have fallen during the refractory period, which would account for the contraction height being more nearly that of the spontaneous contractions *e* and *f*.

Figure 5 shows the production of extrasystoles by single induced shocks applied during late and early diastole to an abdominal heart preparation (type B) when the electrodes were on the dorsal diaphragm over the heart in the fifth abdominal segment

and the lever over the heart in the fourth abdominal segment. The strength of the stimulus was not sufficient to cause interfering contractions of the body muscles. Summations of contractions are

shown, particularly at *f* and *h*, when the shocks *e* and *g* fell at the height of the contraction curve.

COMPENSATORY PAUSE

No recognizable compensatory pauses have been observed to follow the extrasystoles produced in these experiments.

TETANUS OF THE HEART

Figure 6 shows the result of applying to the completely isolated single fourth abdominal segment (preparation D) a series of single

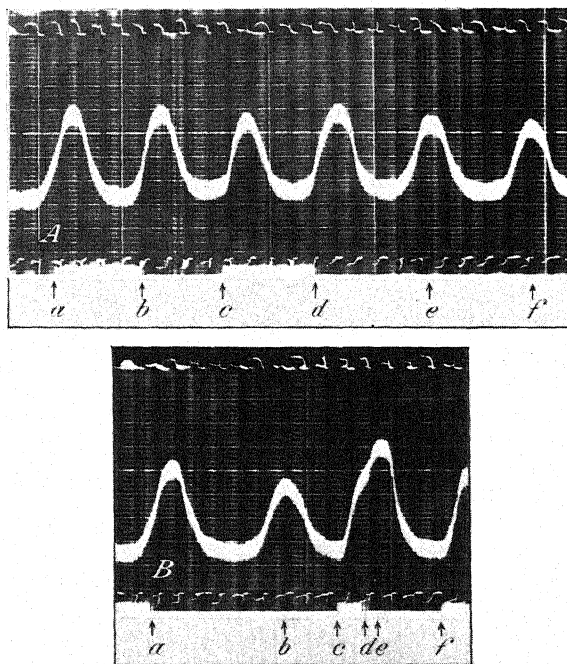


FIGURE 4.—Mechanocardiograms from a fourth abdominal segment (preparation D). Room temperature 29° C.; perfusion with magnesium saline; lever over heart; electrodes in medial position over one alary-muscle group; single induced shocks applied; time in seconds. *A*, Single induced shocks were applied during systole at *a*, *b*, and *d*. The shock at *c* fell either at the end of diastasis or at the beginning of systole; *e* and *f* are spontaneous beats. *B*, Shocks *a* and *d* fall respectively in early and very late systole; *c* and *f* fall during diastasis; *b* is a spontaneous contraction; *e* is a marked summation of contractions.

induced shocks at gradually decreasing time intervals when both the lever and the electrodes are in contact with the dorsal diaphragm over the cardiac tube. *B* is a continuation of *A*, and *C* of *B*. At figure 6, *A*, *d*, summation effects appear and increase until, at *e*, a complete tetanus is developed, which persists until the cessation of stimuli at *B*, *f*. In *B*, between *f* and *g*, there occurs a rapid relaxation, followed by a quick appearance of spontaneous rhythm in which the systoles appear to increase gradually in magnitude as further apparent relaxation occurs. At *C*, *l*, complete recovery has apparently been made.

In the same way, in figure 7, *A* and *B* show cardiac tetanus produced by similarly applying a series of single induced shocks to the abdominal heart preparation (type B) when the lever was on the dorsal diaphragm over the heart in the fifth abdominal segment and the electrodes were over the lateral extremity of the insect's left alary-muscle group. Cardiac tetanus begins at *A*, *e*, lasts to the cessation of stimulation at *B*, *f*, and is followed by rapid relaxation from *f* to *g*. This is followed by a period of inhibition, broken by the spontaneous contractions *h*, *i*, and *j*, of increasing magnitude. Complete recovery is evident at *j*. The record of tetanic contraction of the heart, seen from *A*, *e*, to *B*, *f*, is not a smooth curve, as in figure 6, *A*, *e*, to *B*, *f*, but has superimposed upon it a series of irregularities the

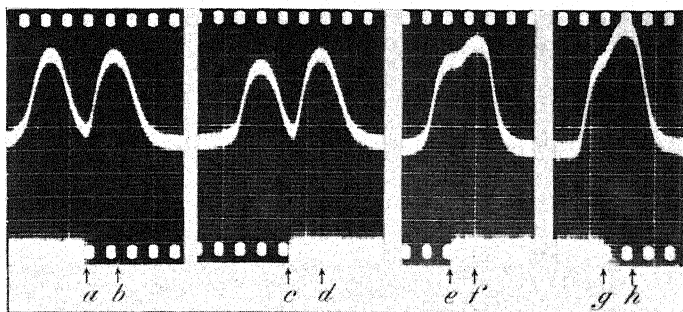


FIGURE 5.—Mechanocardiogram from an isolated abdominal preparation (type B). Room temperature 25° C.; heart preparation kept moist with 0.11 Levy's saline; lever over heart in fourth abdominal segment; electrodes over heart in fifth abdominal segment; time in seconds. Single induced stimuli applied at *a*, *c*, *e*, and *g* produced extrasystoles *b*, *d*, *f*, and *h*, respectively. The shocks were applied during late and early diastole.

larger of which are produced by interfering contractions of the dorsal body muscles given in response to the applied series of stimuli. The minor variations are like those seen in *A*, *a* to *b*, and were caused by mechanical vibrations of the apparatus that had nothing to do with tissue response.

In figure 7, *C*, is shown the first part of a cardiac tetanus similarly produced by stimulating an entire isolated heart preparation (type A). The marked interference of body-muscle contractions with the record of cardiac tetanus is shown.

STAIRCASE EFFECT

Apparent staircase phenomena have been observed, especially when spontaneous cardiac rhythm begins after the temporary inhibition following cardiac tetanus (fig. 6, *B*, *C*, and fig. 7, *B*), and when heart preparations begin spontaneous contractions after certain other periods of diastolic standstill not associated with cardiac tetanus.

REFRACTORY PERIOD

Results of the application of stimuli during various parts of systole (fig. 4) seem to indicate that the stimuli were effective during the greater portion of the contraction period and therefore, if the absolute refractory period occurs, it is confined to at least the early part of systole.

MECHANICAL STIMULATION OF THE HEART

Microscopic observations showed that when the dorsal diaphragm of an isolated heart preparation was mechanically stimulated in the region of the cardiac tube by touching it firmly (but without injury to the tissues) with the point of a dissecting needle, the response of the cardiac tube was a more or less sustained contraction. The con-

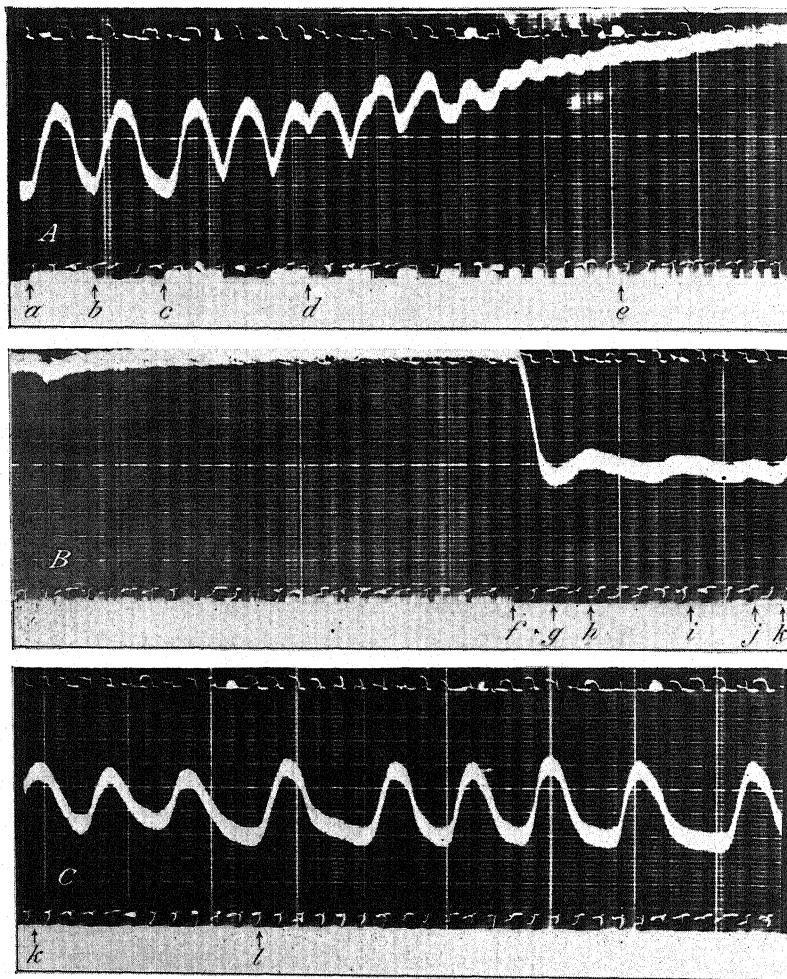


FIGURE 6.—Mechanocardiogram from a completely isolated fourth abdominal segment (preparation D). Room temperature 29° C.; perfusion with magnesium saline; lever and electrodes over heart; time in seconds. *A*, Effects of a series of single induction shocks applied with decreasing time intervals at *a*, *b*, *c*, *d*, and so on. At *e*, complete tetanus is produced. *B*, Continuation of *A*. Stimuli cease to be applied at *f*, and relaxation is completed at *g*; at *h*, *i*, and *j*, incomplete spontaneous beats occur; *k* is part of the first beat *k* shown in *C*. *C*, Continuation of *B*. At *l*, recovery is complete.

traction did not always involve the entire cardiac tube but often was confined to the cardiac regions close to the point of stimulation. This localized contraction has been observed to persist while the spon-

taneous rhythm of other regions of the heart continued without interruption. Whether the contraction was of the nature of a cardiac tetanus, a cardiac contracture, or a different type of response is not known.

In these experiments, isolated heart preparations have often temporarily ceased to exhibit spontaneous rhythm apparently because of mechanical stimuli applied in the process of adjusting the contact

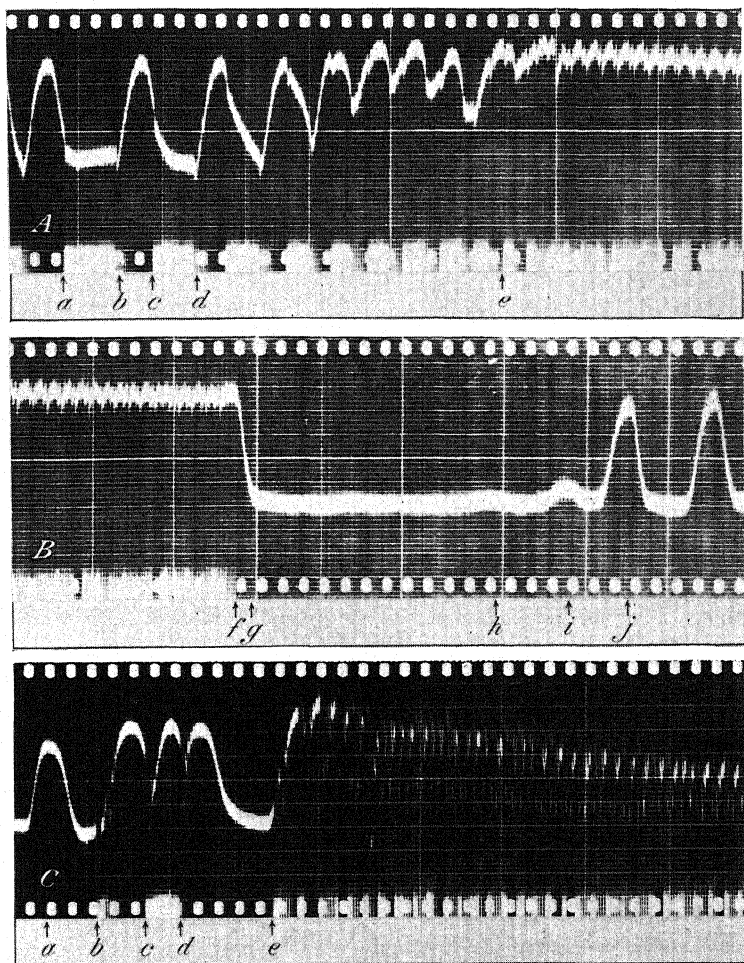


FIGURE 7.—*A* and *B*, Mechanocardiogram from an isolated abdominal heart preparation (type *B*). Room temperature, 25° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode on distal extremity of insect's left fifth abdominal alary-muscle group; time in seconds. *B* is a continuation of *A*. Single induced shocks applied at *a*, *b*, *c*, and so on, with decreasing intervals. At *e*, complete cardiac tetanus is produced, but the curve is disturbed by dorsal body-muscle contractions. The minor vibrations (as from *a* to *b*) are mechanical and have nothing to do with tissue response. *C*, Mechanocardiogram from an entire heart preparation (type *A*). Single induced shocks applied at *a*, *b*, *c*, and so on, and cardiac tetanus produced at *e*. The tetanus curve is disturbed by contractions of dorsal body muscles. Time in seconds.

between lever and dorsal diaphragm. Under these conditions the cardiac response was very probably the same as the response observed to follow noninjurious mechanical irritation with a dissecting needle.

CONTRACTIONS OF THE DORSAL BODY MUSCLES

Figure 8, *A*, shows the effect of repeatedly stimulating an isolated abdominal heart preparation (type B) kept moist with Lévy's saline, when the lever contact was over the heart in the fifth abdominal segment and the electrodes were on lateral extremities of a fifth abdominal alary-muscle group. The paper speed was low. The downward trend of the contractions shown in *A* between *c* and *d* was caused by interfering body-muscle contractions. The single shocks applied at *a*

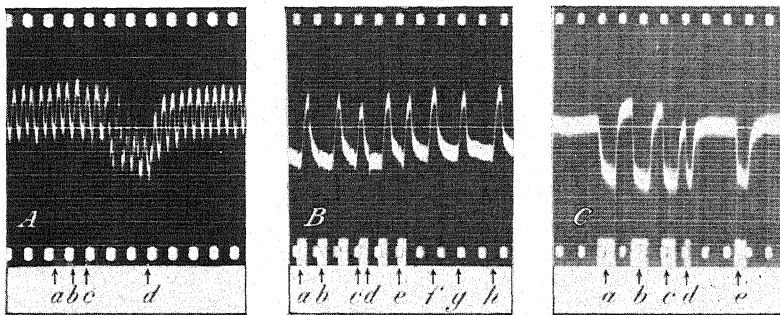


FIGURE 8.—*A* and *B*, Mechanocardiograms from an isolated abdominal heart preparation (type B). Room temperature 29° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode over lateral extremity of alary-muscle group in fifth abdominal segment. *A*, Single induced shocks at *a* and *b*, and a series of single shocks applied between *c* and *d*. *B*, Single induced shocks applied at *a*, *b*, and so on to *e*, with make shock applied at *c* and break shock applied at *d*, and so on. The disturbing effects of dorsal body-muscle contractions are shown. At *f*, *g*, and *h*, are undisturbed spontaneous beats. The stimulating current did not affect the heart, which continued to contract spontaneously. *C*, Record from same preparation after the heart had been severed longitudinally and the fourth, fifth, and sixth left alary-muscle groups removed. Lever medially and electrodes laterally in region where left alary-muscle group had been. Induced tetanizing shocks applied at *a*, *b*, and so on to *e*. Contractions of the dorsal body muscles depressed the curve during stimulation.

and *b* produced no marked effects in this record made with a low paper speed. In figure 8, *B*, are shown the effects of applying single induced shocks to the same preparation at different times in the cardiac cycle when greater paper speed was used. The interference of body-muscle contractions with the mechanocardiogram at the times of stimulation is evident. All the cardiac contractions are spontaneous, and those at *f*, *g*, and *h* exhibit no body-muscle effects.

The dorsal diaphragm and the cardiac tube of this preparation were then severed with a safety-razor blade and the fourth, fifth, and sixth left alary-muscle groups and associated dorsal diaphragm removed. The lever and electrodes were then placed in contact with the exposed dorsal body muscles of the left side, the lever medially and the electrodes laterally. The record thereupon obtained by faradic tetanizing stimulation is shown in figure 8, *C*. The stimuli, applied at *a*, *b*, *c*, *d*, and *e*, resulted in marked depressions of the otherwise straight-line

record. After the removal of the entire heart and the right alary-muscle groups, stimulation gave results similar to those shown in figure 8, *C*.

Figure 9 shows distortions of the mechanocardiogram caused by dorsal body-muscle contractions made in response to single induced

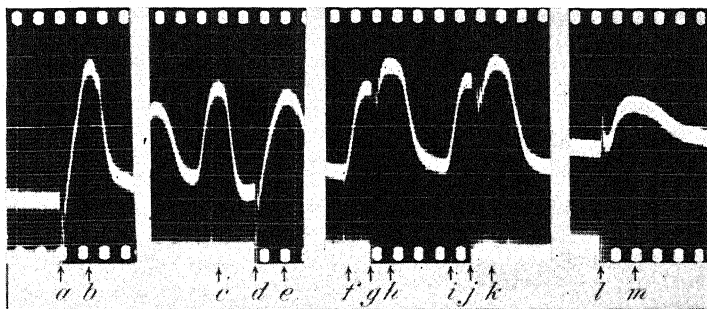


FIGURE 9.—Mechanocardiograms from a whole-heart preparation (type A). Lever and electrodes in an abdominal segment. Single induced shocks applied at *a*, *d*, *g*, *j*, and *l*. At *b*, *e*, *h*, *k*, and *m*, extrasystoles were thus produced. At *h* and *k*, summation effects are recorded. At the time of the stimulus distortions of the curve by dorsal body-muscle contractions can be seen. The upward deflection, at *l*, is unusual; dorsal body-muscle deflections were usually downward. Time in seconds.

shocks applied to the whole heart preparation (type A). Lever and electrode contacts were in an abdominal segment. The contractions *b* and *e* were given in response to stimuli applied at *a* and *d*, respectively, and were preceded by interfering body-muscle contractions

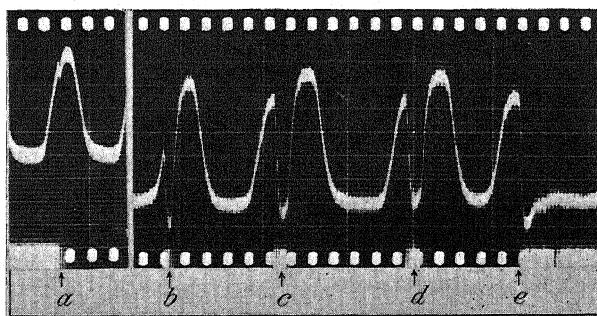


FIGURE 10.—Mechanocardiograms from an isolated abdominal heart preparation (type B). Room temperature 25° C.; heart preparation kept moist with 0.11 Lévy's saline; lever over heart in fifth abdominal segment; electrode over distal extremity of insect's left fifth abdominal alary-muscle group; time in seconds. At *a*, a single induction shock was applied. At *b*, *c*, and *d*, short tetanizing stimuli were applied. At *e*, a more prolonged tetanizing stimulation was begun. The marked effects of dorsal body-muscle contractions on the mechanocardiograms are shown.

whose records somewhat resemble presystolic notches. Similar distortions, however, occurred during other parts of the cardiac cycle at *g*, *j*, and *l*. Usually the body-muscle contractions produced depressions of the curve, but at *l*, the deflection was upward.

Figure 10 shows similar results of applying short tetanizing stimuli to an abdominal heart preparation (type B). The lever was over the heart in the fifth abdominal segment, and the electrodes were over the lateral extremities of the left alary-muscle group of the same segment. The very small vibrations were mechanical and had nothing to do with tissue response. Marked disturbances of the heartbeat record are shown.

Figure 11 shows depressions of an otherwise straight-line record obtained from an incompletely isolated abdominal segment (preparation C) from which the heart and both alary-muscle groups had been removed. The lever was in the region where the heart had been, and the electrodes were placed laterally where one of the alary-muscle groups had been located. Series of single induced shocks were applied between *a* and *b* and between *c* and *d*. During stimulation the otherwise straight-line record was depressed by contractions of the dorsal body muscles. The external dorsal body muscles are considered to be responsible for this movement.

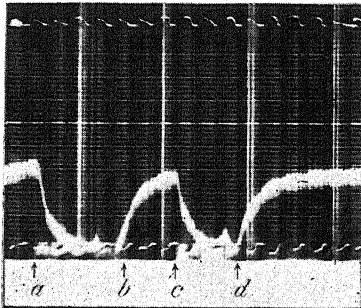


FIGURE 11.—Mechanocardiogram from an incompletely isolated third abdominal segment (preparation C) about 5 hours old. Room temperature 29° C.; preparation perfused with the magnesium saline; time in seconds. This record was obtained after cardiac tube and both alary-muscle groups had been removed. Lever on midregion where cardiac tube had been; electrodes lateral to lever, in region where one alary-muscle group had been. From *a* to *b* and from *c* to *d*, a series of single induced shocks were rapidly applied. The contractions of the dorsal body muscles depressed the curve.

When the completely isolated third abdominal segment (preparation D) from which the heart and both alary-muscle groups had been removed was similarly stimulated with induced shocks, applied either singly or in rapid succession, or with tetanizing stimuli, no appreciable effect of body-muscle contraction upon the otherwise straight-line record was observed.

SUMMATION OF STIMULI

It was observed that when slightly subminimal single shocks were rapidly applied by manipulating the key of the primary circuit or when tetanizing stimuli of subminimal strength were applied, contractile responses of the heart and of the

dorsal body muscles could be obtained. This indicates that summation of stimuli occurred in these muscles.

ALARY MUSCLES

No convincing evidence was obtained that the application of single induced shocks or of tetanizing stimuli to the alary-muscle fibers caused them to give responsive contractions. When the alary muscles were stimulated while they were under low-power microscopic observation they were not observed to respond with visible contraction either in the form of twitches or of visible tonus changes.

DISCUSSION

It is generally recognized³ that the vertebrate heart (especially frog and mammalian ventricles) can respond to applied stimuli with extrasystoles, particularly when the stimuli are strong and are applied during late diastole or during diastasis. Because it possesses an absolute refractory period that persists throughout the systole and a relative refractory period that extends into at least the earlier portion of diastole, vertebrate cardiac muscle is not responsive to stimulation while it is contracting and responds to only unusually strong stimuli while it is relaxing, especially during early diastole. For the same reasons and because it exists in syncytial form, vertebrate cardiac muscle does not normally exhibit well-defined summation of contractions in response to single shocks or complete tetanic contractions when stimulated with tetanizing shocks. It can display staircase phenomena, compensatory pauses, and response to mechanical as well as to electrical and other stimuli. Its response to mechanical stimulation, applied by touching the ventricle with the point of a needle, appears as a twitch, not as a sustained contraction.

Very little has been known of the responses to electrical stimulation of the insect heart, especially the isolated insect-heart preparation. From the results of these experiments it is evident that in the perfused isolated-heart preparation of *Periplaneta americana* the cardiac responses differ considerably from the responses usually reported to be given by vertebrate hearts. The cockroach heart can respond to single induced shocks with extrasystoles during both diastasis and diastole. The extrasystolic contractions are more or less summated, the degree of summation being greatest at the height of the contraction curve, that is, at the very beginning of diastole and perhaps at the very end of systole. These results would seem to indicate either (1) that the cockroach cardiac muscle does not react syncytially as does vertebrate cardiac muscle, but behaves more like skeletal muscle, in which the individual fibers, but not the muscle as a whole, give the all-or-none response, or (2) that the cockroach cardiac muscle functions syncytially but fails to obey the all-or-none law and gives graded contractions. They also indicate that at the height of contraction and in early diastole the insect cardiac muscle is not in an absolute refractory state and probably is not in a marked relative refractory state. If, furthermore, the slight increase in contraction height resulting from the application of a single shock during the early, middle, or late portion of systole is to be interpreted as a summation of contractions of the cardiac-muscle fibers⁴ given in response to the stimulus, it would seem that the insect cardiac muscle fails to exhibit absolute refractoriness throughout all except perhaps the very early part of systole.

The cockroach heart gives a well-defined tetanus in response not only to tetanizing stimuli but also to single shocks rapidly applied by opening and closing the key of the primary circuit by hand. In this response the cockroach heart differs from the vertebrate heart. The

³ See various textbooks, manuals, and reviews of vertebrate physiology for prevailing opinions as to the usual characteristics of vertebrate hearts and cardiac muscle, for example, books by Howell, Starling, Bayliss, MacLeod, and others.

⁴ If the increased contraction height of the curve is not caused by additional cardiac-muscle contraction, it seems that it would have to result from the alary-muscle fibers responding to the stimulus with relaxation. Since contractions of the alary-muscle fibers would be expected to depress, and the contractions of the dorsal body muscles have been found nearly always to depress the curve of heartbeat. This would be contrary to the way muscle fibers are now known to respond to stimuli.

cardiac tetanus thus obtained serves as additional evidence that at the height of the contraction curve the insect cardiac muscle is not in a state of absolute refractoriness.

The cockroach heart also differs from the vertebrate heart in that no recognizable compensatory pauses have been found to follow extrasystoles. Although occasionally certain extrasystoles have been followed by diastatic periods somewhat longer than those preceding the same extrasystoles, the observed increase of diastasis is not significantly great when the variations in duration of diastasis that commonly occur spontaneously are considered. The lack of occurrence of compensatory pauses by the cockroach heart suggests that stimulating this heart is analogous to stimulation not of the ventricle but rather of the sinus venosus, for example, of the frog heart.⁵ If this analogy is correct, it implies that in stimulating the cockroach heart, as was done in these experiments, the stimuli act upon something corresponding to pacemaker tissue or to a tissue that sets its own pace. This would not be unexpected where, as in most of these experiments, a strong stimulus is applied directly to the cardiac muscle of an isolated segment of the heart mechanism.

Comparison of the results obtained with the different types of isolated heart preparation used in these experiments shows that movement of the preparation resulting from contractions of the dorsal body muscles is a factor that must be brought into consideration when experiments are undertaken in which insect heart preparations are subjected to various stimuli, either electrically or by the application of certain dissolved substances.⁶ It is evident that one way to eliminate interfering movements of the dorsal body muscles is to utilize preparation D (see Methods) in which the internal and external dorsal body muscles are either severed or torn from their insertions.

The movements caused by contractions of the dorsal body muscles may affect the mechanocardiogram in more than one way, but in these experiments the effect was nearly always to depress the record of heartbeat. With strong single shocks, sharp, rapid, momentary depressions at the time of stimulus are superimposed on the mechanocardiogram and, when occurring just before systole, may somewhat resemble the presystolic notch. This resemblance may be quite marked when the stimulus is a very short tetanizing current. The fact that through stimulation the body-muscle depressions can be made to occur at other times during the cardiac cycle than just prior to systole indicates the difference between these depressions and the spontaneously occurring presystolic notches. This is shown also by comparison of the forms of the body-muscle depressions and the presystolic notches. It is hardly to be expected that dorsal body-muscle contractions are the cause of the presystolic notch since this would have the improbable meaning that the dorsal body muscles contract rhythmically in exact synchronism with the heart rhythm. The origin of the presystolic notch, therefore, is probably to be sought among other causes.

⁵ A compensatory pause often follows an extrasystole of the frog heart produced by stimulation of the ventricle. The pause is thought to be caused by the next excitatory impulse from the sinus venosus (pacemaker) reaching the ventricle when the latter is in the refractory state produced during the extrasystole. The ventricle has to wait for still another excitatory impulse to arrive from the pacemaker before it contracts. Stimuli applied to the sinus venosus (pacemaker), therefore, produce extrasystoles that are not followed by compensatory pauses.

⁶ Since nicotine can produce contraction or contracture in skeletal muscle (Σ), its effect upon insect dorsal body muscle deserves consideration, particularly when high or toxic concentrations are used.

In none of these experiments has evidence been obtained that the applied stimuli produced recognizable contractions of the alary-muscle fibers. It is possible that the alary-muscle fibers contracted when stimulation caused interfering contractions of the dorsal body muscles, but when these interfering movements were not produced, the alary muscles exhibited no signs of undergoing contraction, even when the stimulating electrodes were over the alary-muscle fibers themselves and the stimulus was strong enough to cause contraction of the cardiac muscle. Neither did the alary-muscle fibers show any recognizable shortening when observed microscopically during stimulation. Although this evidence does not constitute proof that the alary-muscle fibers are incapable of contraction, it does render more probable the hypothesis that the alary-muscle fibers of *Periplaneta americana* tend to maintain a tonus and to apply a steady pull upon the wall of the cardiac tube rather than to produce diastole by rhythmic twitches given synchronously with the rhythm of the cardiac muscle. These facts would also indicate that even should contractions of the alary-muscle fibers occur during, and be undetected because of, interfering body-muscle contractions, the threshold of excitability of the alary-muscle fibers is considerably higher than that of the cardiac muscle in the same abdominal segment.

The failure to obtain recognizable contraction of the alary-muscle fibers in these experiments also lends weight to the hypothesis (4) that the presystolic notch results not from the rhythmic presystolic contraction of the alary-muscle fibers but probably from a presystolic increase of intracardiac hydrostatic pressure resulting from the contraction of the heart in some segment other than that from which the mechanocardiogram is being recorded.

These isolated heart preparations no doubt contained intrinsic nervous mechanisms (5) that probably have to do with cardiac regulation. What effect these structures had in the response of the heart to stimulation in these experiments is not known, but, since leakage of electrical current through the perfusion saline from one electrode to the other necessitated the use of strong stimuli, it was assumed that the cardiac, alary, and dorsal body muscles were subjected to direct stimulation.

SUMMARY AND CONCLUSIONS

Four different types of isolated heart preparations from the American cockroach (*Periplaneta americana* (L.)) have been electrically stimulated and mechanocardiograms of their responses recorded. The four types were: Type A, the whole isolated heart preparation, including both thoracic and abdominal portions; type B, the abdominal heart preparation; type C, the single abdominal segmental preparation, incompletely separated from the adjacent margin of the adjacent overlapping segment but completely separated from the adjacent overlapped segment; and type D, the single abdominal segment completely separated from both adjacent segments. The preparations were occasionally flooded or continuously perfused with saline solutions.

The applied stimuli consisted of single induced shocks, induced shocks rapidly repeated by opening and closing the key of the primary circuit by hand, and induced tetanizing shocks consisting of a series of

shocks applied at the rate determined by the vibrator of the inductorium.

The mechanocardiograms obtained show that the heart of this insect can respond to single shocks, applied during diastasis and diastole, with extrasystoles that are more or less summated, particularly during early diastole; that it gives an apparent summation response when the shock falls during systole; and that the extrasystoles thus obtained are not followed by compensatory pauses.

It is also shown that the heart can respond to tetanizing stimulation with a complete cardiac tetanus and can rapidly relax and quickly its original spontaneous rhythm after the cessation of stimulation. Posttetanic standstills often occur.

During recovery from posttetanic standstill and during recovery from standstill produced by certain other causes, the cockroach heart exhibits apparent staircase phenomena.

The responses of this heart to single shocks applied at different times in the cardiac cycle and to tetanizing stimuli show that this insect's cardiac muscle is not absolutely refractory during diastole, diastasis, and, probably, late systole. They also indicate that, if the absolute refractory period exists in this cardiac muscle, it is probably confined to early systole.

This insect heart can respond to mechanical stimulation with a more or less prolonged contraction, which may be localized in that part of the heart tube lying near the point of stimulation.

Contractions of the dorsal body muscles may cause variations to be superimposed on the heartbeat record. The variations may stimulate the presystolic notches when they are caused by shocks eliciting extrasystoles or when they happen to fall immediately prior to systoles.

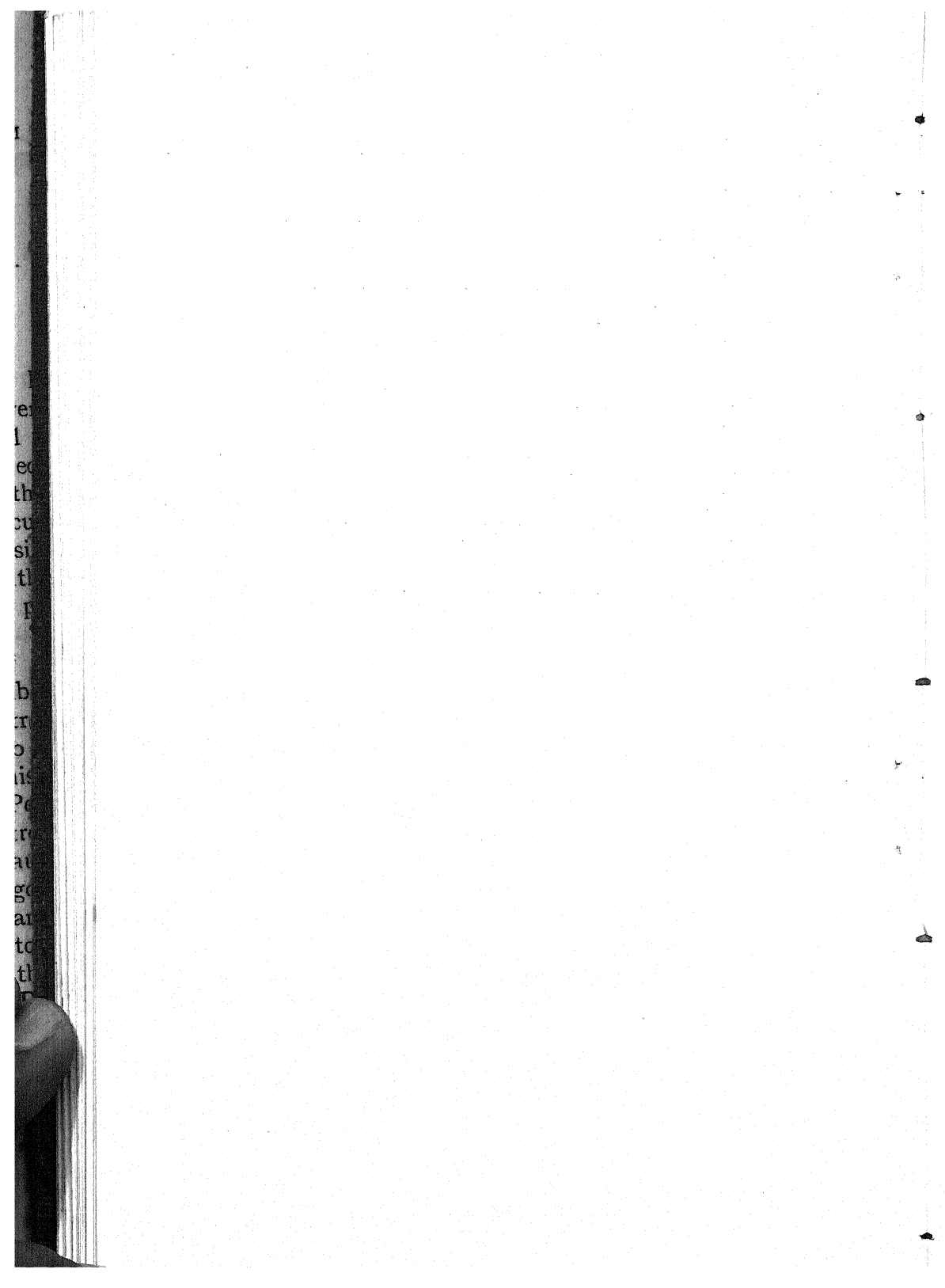
The heart of this insect differs from the vertebrate heart (particularly frog and mammalian ventricles) in its ability to respond to stimulation with summated extrasystolic contractions, in its failure to exhibit compensatory pauses, in its apparently shorter absolute refractory period, and in its ability to respond to a mechanical stimulus with a more or less prolonged contraction.

Stimulation of the alary-muscle fibers (of the heart) with strong single, repeated, or tetanizing shocks yielded no evidence that they responded with recognizable contractions in the form of either twitches, tetanus, or tonus changes. These negative results lend weight to the hypothesis that cardiac dilation during diastole is not produced by rhythmic contractions of the alary muscles but rather that the latter tend to exert a steady tension on the cardiac walls. They also support the view that the presystolic notch is produced not by presystolic contractions of the alary-muscle fibers but by some other agency (probably increases of intracardiac hydrostatic pressure).

Interfering contractions of the internal and external dorsal body muscles may interfere with mechanocardiograms obtained from heart preparations of types A, B, and C. These can be eliminated by using the type D preparation.

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JELLYING AND CRYSTALLIZATION OF SIRUPS MADE FROM DIFFERENT PARTS OF THE SORGO STALK AT DIFFERENT STAGES OF MATURITY¹

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EARLIER WORK

The fact that starch is present in sorgo juices has been known for many years. In 1858 Gilbee (3)² was granted a British patent on a process for eliminating organic impurities from the juices of sacchariferous plants by the use of alcohol. In 1864 Joulie (5), in recommending a method of clarification by the use of alcohol, mentioned that the alcohol precipitate contained starchy materials, and suggested conversion to sugar for production of alcohol. Sylvester (10) found the starch granules in sorgo juice to be from one eight-thousandth to one six-thousandth of an inch in diameter, as compared with one five-hundredth to one four-hundredth of an inch for granules of potato starch. Hutchinson (9), in discussing the distribution of starch in the sorgo stalk, reported that the iodine test showed no starch in green canes. If any blue color was present it was obscured by an intense brown coloration. With well-matured canes iodine gave an intensely blue color toward the top, which decreased in intensity toward the butt. At the beginning of maturity, as indicated by the presence of sucrose in the lower part of the stalk, more starch was found in the butt than in the top. Janssen, McClelland, and Metzger (4) in reporting analyses of sorgo juices from the different internodes of the Honey variety stated that starch occurs throughout the internodes of the stalk, although it is highest in the middle internodes.

Sherwood (6, 7) reported the starch content of the juices of 15 varieties of sorgo and proved that starch is the cause of jellying or clabbering of sorgo sirups. Willaman and Davison (11), in studying the jellying of sorgo sirups, found that 0.32 to 1.15 percent of starch is present in normal sirups and 1.17 to 3.36 percent in sirups that jelly. Walton and Ventre³ found that starch is responsible for slow boiling and scorching in the evaporator, and devised a farm-scale method for preventing this condition.

Many investigators have attempted to develop a practicable process for utilizing sorgo as a commercial source of sugar (sucrose). Among those who also studied the dextrose and levulose content of the stalk were Berthelot and Trannoy (2), who reported analyses of sorgo juices at eight stages of plant maturity from August 10 to November 30. At each stage of maturity the juices contained from approximately

¹ Received for publication March 17, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 150.

³ WALTON, C. F., JR., VENTRE, E. K., and BYALL, S. HOW TO PREVENT SLOW BOILING, SCORCHING, CLABBERING, AND SUGARING OF SORGHUM SIRUP. U. S. Bur. Chem. and Soils Cir. 6 pp. 1935. [Mimeo-graphed.]

two to four times as much dextrose as levulose, and at the two earliest stages the dextrose content exceeded the sucrose content. It appears, however, that these earlier investigators did not study the crystallization characteristics of sirups made from the stalk as a whole in comparison with those of sirups made from different parts of the stalk. Moreover, it must have been assumed that the sugar content of the sorgo stalk is similar to that of sugarcane. No report has been found of dextrose crystallization in sorgo sirups.

On the whole the literature indicates that earlier investigators made little attempt to correlate composition of the juice and quality of the sirup, especially the effect of the content of starch, sucrose, and dextrose in the juice on the composition and properties of the resulting sirups.

In the manufacture of sorgo sirups by the customary methods, the effect on the starch and sucrose of continued heating in a slightly acid solution should produce a somewhat greater proportion of dextrose in the sirups than in the juices. Since both the content of starch and sugars in different parts of the stalk and the effect of maturity on the composition of the juice must be considered, it is obvious that farm-made sorgo sirups present a special case of the system sucrose, dextrose, and levulose in a relatively impure solution containing also varying proportions of starch and its degradation products produced by the action of heat and acidity during open evaporation. The purpose of the present investigation was to determine the effect of the sugar and starch content of sirups in relation to crystallization and jelling. Sirups made from different parts of the stalk at different stages of maturity were compared with those made from the whole stalk. Inasmuch as the sirups were made from different portions of the same stalk, an opportunity was afforded to study jelling and crystallization under uniform agronomic conditions.

EXPERIMENTAL PROCEDURE

In continuation of previous work by the authors (12) an investigation was conducted in cooperation with the Mississippi Agricultural Experiment Station⁴, the four varieties of sorgo (*Holcus sorghum* var. *saccharatus*) most used for sirup production in that section being selected for study. These varieties, designated by the names locally assigned to them, had the following varietal characteristics:

Hodo.—A large-barrel, long-stalk, late-maturing variety.

Iceberg.—A small-barrel, short-stalk, early-maturing variety.

Honey.—A small-barrel, medium-height stalk, intermediate-maturing, sprangle-top variety.

Gooseneck.—A medium-barrel, medium-height stalk, intermediate-maturing variety.

The stages of maturity were determined by the average maturity of the seed heads, described as follows:

Milk stage.—When the seed heads were out of the sheath and well formed, and the contents when pressed out had the consistence of milk.

Dough-to-ripe stage.—When the contents pressed from the seed heads had the consistence of stiff dough, the stage just preceding the hard-dough stage.

Dead-ripe stage.—When the contents of the seed heads were solid, and the glumes were wide open and dry.

⁴The authors wish to express their appreciation for assistance given by the late J. R. Ricks, director, and by W. R. Perkins, formerly assistant director, Mississippi Agricultural Experiment Station, in supplying working facilities and in connection with the agronomic work of this investigation.

When the sorgo had reached the selected stage of maturity, the stalks were cut even with the surface of the ground and stripped of leaves. The seed head and peduncle were removed, and the stalks divided at the nodes. The internodes were numbered consecutively from the peduncle and segregated in batches according to number. Each batch was ground separately in a horse-driven farm mill, being passed through the mill twice in order to extract as much juice as possible with this type of milling equipment.

A whole-stalk sample of each variety at each stage of maturity was used for comparison. An aliquot portion of the stalks was selected and stripped clean, after which the seed heads and peduncle were removed. This sample was subjected to exactly the same treatment as the various batches of internodes.

The juices expressed separately from each of two consecutively numbered batches of internodes were combined, and this juice was made into sirup for comparison with the sirup made from the whole stalk. The experimental method developed by this Division (8) for producing farm-made sirups was used in making all the sirups. Samples of the sirups were taken in triplicate and sealed while hot in 4-ounce, screw-top, glass jars. One sample was used for analysis; the other two were stored in a constant-temperature room at 20° C. for about 2 years. The sirups were made and analyzed in the fall of 1935, and the final examination for jellying and crystallization was made in February 1938.

The analytical determinations were made as follows:

Total solids.—Determined directly on the sirups at 20° C. by the use of the Zeiss sugar refractometer.

Sucrose.—Determined by the Association of Official Agricultural Chemists method of double polarization (1), invertase being used as the inverting reagent.

Reducing sugars.—Determined by the Munson and Walker method (1) and calculated in terms of invert sugar.

Starch was determined by the authors' modification of the official A. O. A. C. method (1) of starch determination in the presence of interfering polysaccharides. This method may be described briefly as follows:

For the malt reagent grind well-cleaned, new barley malt of high diastase activity and prepare an infusion of this freshly ground malt, just before it is to be used, by digesting 5 gm. with 100 cc. of water at room temperature for 2 hours, or for 20 minutes if the mixture can be stirred by an electric mixer. Filter to obtain a clear extract, if necessary returning the first portions of the filtrate to the filter. Mix the infusion well.

Wash a 15-gm. portion of the sirup into a beaker with approximately 300 cc. of 70-percent alcohol (by volume) and thoroughly mix by stirring.

Wash the precipitated starch free of sugar with additional 70-percent alcohol by decantation through an alundum crucible (porosity R. A. 360). Return the crucible to the beaker containing the washed starch precipitate and dry in an oven at 105° C. until free of alcohol. Then add 160 cc. of hot water and thoroughly gelatinize the starch at boiling temperature in a water bath. (At this point start a control to determine the quantity of dextrose derived from the malt reagent. This is done by adding 160 cc. of distilled water to a 400-cc. beaker and following the same procedure used for the starch determination.)

Cool to 50° C. or lower. Add 20 cc. of malt extract reagent and place in a temperature-controlled water bath. Keeping the mash thoroughly mixed, gradually raise the temperature to 70° in 20 to 30 minutes. Maintain at 70° for 30 minutes, stirring from time to time; then increase the temperature to 80° and keep it at that temperature for 10 minutes. Finally heat to the boiling point. Keep the mixture well stirred. Cool the contents of the beakers and the water bath to 55°. Add another 20 cc. of the malt-extract reagent, mix well, and hold at 55° for 1 hour, stirring about once every 10 minutes. At the termination of the digestion increase the temperature rapidly to above 80°.

Transfer to a 500-cc. volumetric flask and add 316 cc. of 95-percent alcohol, a little at a time, shaking between additions. Wash the crucible and beaker with hot water and use the washings to make up to the mark. Filter through a dry filter paper and evaporate the filtrate to a volume of 15 to 20 cc. or until free from alcohol.

Transfer the aqueous residue to a 200-cc. volumetric flask. Wash the beaker with hot water, using a rubber-tipped rod to recover any dextrose present, and add the washings to the volumetric flask. Allow it to cool, and then complete the volume to 200 cc. Transfer the contents to a suitable digestion flask, add 20 cc. of hydrochloric acid (specific gravity 1.125), made by diluting 68 cc. of strong acid (sp. gr. 1.19) to 100 cc., and connect the flask with a reflux condenser. Heat in a boiling water bath for 2½ hours. Cool and transfer to a 250-cc. volumetric flask. Partly neutralize, while stirring, by adding 10 cc. of strong caustic soda solution (44 gm. NaOH per 100 cc. of H₂O), and complete neutralization with a little anhydrous Na₂CO₃. Cool to room temperature and make up to the 250-cc. mark.

Determine the dextrose in a 50-cc. aliquot. Correct the weight of dextrose obtained by subtracting the weight of dextrose obtained in a 50-cc. aliquot of the malt reagent control. Multiplying this value by 0.9 gives the weight of starch in the 50-cc. aliquot. This value $\times \frac{1}{5} \times 100 =$ the percentage of starch in the sirup.

EXPERIMENTAL RESULTS

Tables 1, 2, 3, and 4 give the sucrose, reducing sugars, and starch content of the sirups, and the calculated amounts of each in grams per 100 gm. of water as determined by the refractometer. The tables also show the ratio of sucrose to reducing sugars, the ratio of reducing sugars to sucrose, and the type of crystallization and jellying, if any.

TABLE 1.—Solids, sucrose, reducing sugars, and starch content of sirups made from various internodes of Gooseneck variety of sorgo at different stages of maturity

MILK STAGE

Sirup No.	Inter-nodes	Total solids ¹	Water ¹	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization		Starch			Jellying
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose			On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	
	Nos.	Percent	Percent	Percent	Grams	Grams	Percent	Grams	Grams			Dextrose	Dextrose	Percent	Grams	Grams	Negative.
1	1-2	75.30	20.70	20.43	78.26	253.38	52.45	66.13	253.38	0.30	3.23	do	do	0.39	0.50	1.91	Negative.
2	3-4	75.30	20.70	19.38	77.83	203.09	50.57	67.33	203.09		2.61	do	do	0.27	0.36	1.08	Do.
3	5-6	76.60	21.40	25.80	102.57	242.52	51.90	66.02	242.52	0.42	2.36	do	do	0.23	0.30	1.10	Do.
4	7-8	77.90	22.10	27.92	107.87	244.57	54.03	69.37	244.57	0.35	2.78	do	do	0.19	0.25	0.88	Do.
5	9-10	76.50	23.50	7.52	24.46	280.63	65.95	86.20	280.63	0.08	11.46	do	do	0.16	0.21	0.70	Do.
6	11-12	74.10	25.90	5.72	16.37	249.03	64.50	87.03	249.03	0.06	15.21	do	do	0.07	0.09	0.27	Do.

DOUGH-TO-RIPE STAGE

Sirup No.	Inter-nodes	Total solids ¹	Water ¹	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization		Starch			Jellying
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose			On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	
	Nos.	Percent	Percent	Percent	Grams	Grams	Percent	Grams	Grams			Dextrose	Dextrose	Percent	Grams	Grams	Negative.
7	1-2	73.70	26.30	46.63	130.68	113.30	29.80	40.43	113.30	1.15	0.86	Negative	Negative	0.44	0.60	1.68	Negative.
8	3-4	79.30	20.70	50.40	103.09	157.72	32.65	41.17	157.72	1.22	.81	Sucrose	Sucrose	.39	.50	1.91	Do.
9	5-6	74.70	25.30	39.71	117.27	157.70	39.90	53.41	157.70	.74	1.34	do	do	.28	.38	1.12	Do.
10	7-8	72.10	27.90	27.93	72.18	172.90	48.24	66.90	172.90	.41	2.39	do	do	.16	.22	.56	Do.
11	9-10	76.70	23.30	21.71	71.45	240.81	56.11	72.12	240.81	.29	3.37	do	do	.09	.12	.39	Do.
12	11-12	78.30	21.70	21.07	76.03	260.96	56.63	72.32	260.96	.29	3.43	do	do	.08	.11	.39	Do.
13	(²)	75.50	24.50	44.31	136.57	150.20	36.80	48.74	150.20	.91	1.10	do	do	.20	.27	.83	Do.

DEAD-RIPE STAGE

Sirup No.	Inter-nodes	Total solids ¹	Water ¹	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization		Starch			Jellying
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose			On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	
	Nos.	Percent	Percent	Percent	Grams	Grams	Percent	Grams	Grams			Dextrose	Dextrose	Percent	Grams	Grams	Negative.
14	1-2	75.80	24.20	54.52	170.78	98.26	23.78	31.37	98.26	1.74	0.57	Negative	Negative	0.86	1.14	3.57	Jelled.
15	3-4	76.90	23.10	49.48	191.98	108.13	24.98	32.48	108.13	1.77	.56	do	do	.83	1.08	3.59	Do.
16	5-6	77.00	23.00	38.30	166.52	138.26	31.80	41.29	138.26	1.20	.83	Sucrose	Sucrose	.77	1.00	3.54	Do.
17	7-8	73.70	24.30	43.75	136.41	147.09	35.89	47.36	147.09	.92	1.08	Negative	Negative	.57	.76	2.86	Do.
18	9-10	74.50	25.50	30.82	116.35	170.64	43.49	58.53	170.64	.68	1.46	Dextrose	Dextrose	.43	.58	1.69	Negative
19	11-12	72.70	27.30	34.15	90.95	149.49	40.81	56.13	149.49	.60	1.64	do	do	.42	.58	1.54	Do.
20	(²)	74.10	25.90	50.46	144.40	119.49	30.95	41.76	119.49	1.21	.83	Negative	Negative	.67	.91	2.60	Jelled.

¹ By refractometer.² Whole stalk.

TABLE 2.—Solids, sucrose, reducing sugars, and starch content of sirups made from various internodes of Honey variety of sorgo at different stages of maturity

MILK STAGE

Sirup No.	Inter-nodes	Total solids †	Water †	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystalliza-tion	Starch			Jellying	
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose		On sirup basis	Per 100 gm. of solids	Per 100 gm. of water		
	Nos.	Percent	Percent	Percent	Grams	Grams	Percent	Grams	Grams	Grams	0.62	1.61	Dextrose----	Percent	Grams	Grams	Jellied.
21	1-2	77.60	22.40	24.07	31.03	107.45	38.80	50.01	173.21	.60	1.66	do-----	.82	1.06	3.67	Do.	
22	3-4	80.20	19.80	27.40	34.18	138.38	45.55	56.82	230.05	.37	2.69	do-----	.65	.81	3.28	Do.	
23	5-6	81.40	18.60	20.80	25.67	112.31	56.20	69.07	302.15	.22	4.42	do-----	.49	.61	2.67	Do.	
24	7-8	73.50	26.50	13.02	17.72	49.13	57.55	78.33	217.17	.04	22.83	do-----	.47	.64	1.77	Negative.	
25	9-10	71.60	28.40	2.88	4.02	10.14	65.75	91.82	231.51	.02	48.01	do-----	.39	.54	1.37	Do.	
26	11-12	73.40	26.60	1.36	1.85	5.11	65.30	88.96	245.49	.40	2.50	do-----	.26	.35	.97	Do.	
27	(²)	76.40	23.60	20.28	27.85	85.93	50.85	66.58	215.46					.60	1.94	Do.	

DOUGH-TO-RIPE STAGE

28	1-2	79.50	20.50	35.76	48.75	189.07	29.42	37.00	143.51	1.31	0.76	Negative----	0.52	0.66	2.56	Jellied.
29	3-4	77.30	22.70	40.58	52.49	178.76	31.77	41.09	139.95	1.27	.78	Sucrose----	.51	.66	2.25	Do.
30	5-6	75.90	24.10	34.82	45.87	144.48	38.20	50.32	158.50	.91	1.10	Dextrose----	.66	.87	2.74	Do.
31	7-8	73.50	26.50	25.74	35.02	97.13	44.50	60.54	167.02	.57	1.72	do-----	.55	.75	2.07	Negative.
32	9-10	76.50	23.50	17.11	22.36	72.80	54.95	72.83	233.83	.31	3.21	do-----	.56	.73	2.37	Do.
33	11-12	72.60	27.40	10.60	14.60	38.68	56.15	77.33	204.93	.19	5.29	do-----	.40	.56	1.48	Do.
34	(²)	72.80	27.20	24.22	33.26	89.04	45.25	62.15	166.36	.53	1.86	do-----	.52	.71	1.90	Do.

DEAD-RIPE STAGE

35	1-2	76.90	23.10	24.22	31.49	104.84	40.75	52.98	176.40	0.59	1.68	Negative-----	0.55	0.72	2.40	Jellied.
36	3-4	80.00	20.00	31.95	39.93	159.75	40.50	50.62	202.50	.70	1.26	do-----	.60	.75	3.00	Do.
37	5-6	77.30	22.70	17.87	25.06	82.26	50.92	70.14	174.28	.36	2.78	Dextrose-----	.48	.67	1.66	Negative.
38	7-8	73.30	26.70	22.41	30.57	83.93	48.10	65.61	180.15	.48	2.14	do-----	.74	1.01	2.77	Jellied.
39	9-10	71.20	28.80	5.30	7.44	18.40	62.75	88.12	217.88	.08	11.84	do-----	.66	.82	2.29	Do.
40	11-12	72.30	27.70	5.60	7.74	20.21	63.05	87.19	227.71	.08	11.26	do-----	.60	.83	2.16	Negative.
41	(²)	78.60	21.40	37.40	47.53	174.76	35.47	45.12	165.75	1.05	.95	Negative-----	.80	1.02	3.74	Jellied.

¹ By refractometer.² Whole stalk.

TABLE 3.—Solids, sucrose, reducing sugars, and starch content of sirups made from various internodes of Iceberg variety of sorgo at different stages of maturity

DOUGH-TO-RIPE STAGE

Sirup No.	Inter-nodes	Total solids ¹	Water ¹	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization	Starch		Jellying	
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose		On sirup basis	Per 100 gm. of solids		Per 100 gm. of water
42	No. 2	Percent	Percent	Percent	Grams	Grams	Percent	Grams	Grams	2.43	0.41	Sucrose	Percent	Grams	Grams	Jellied.
43	3-4	81.90	18.10	52.38	289.39	119.06	21.55	26.31	119.06	2.26	0.44	do	0.84	1.03	4.66	Do.
44	5-6	78.30	21.70	51.82	236.49	104.60	22.70	28.99	104.60	1.74	0.57	do	.75	.96	3.45	Do.
45	7-8	77.90	22.10	47.24	213.76	94.87	27.17	34.87	122.94	1.82	.75	do	.61	.66	2.32	Do.
46	9-10	77.40	22.60	41.70	184.91	40.72	31.52	40.72	139.47	1.82	1.21	Dextrose	.49	.64	2.19	Negative.
47	(2)	77.50	22.50	46.33	205.91	33.96	26.32	51.64	116.97	1.76	.57	Sucrose	.44	.59	1.70	Do.
														.86	2.96	Jellied.

DEAD-RIPE STAGE

Sirup No.	Inter-nodes	Total solids ¹	Water ¹	True sucrose (Clerget)			Reducing sugars as invert sugar				Sugar ratios		Crystallization	Starch		Jellying
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose	On sirup basis		Per 100 gm. of solids	Per 100 gm. of water	
48	1-2	71.00	29.00	47.99	165.48	51.45	14.92	21.02	51.45	3.21	0.31	Sucrose	1.15	1.63	3.98	Jellied.
49	3-4	80.00	20.00	55.41	277.05	91.60	18.32	22.91	91.60	3.02	.33	do	1.04	1.30	5.20	Do.
50	5-6	76.80	23.20	49.21	212.11	96.77	22.45	29.24	96.77	2.19	.46	do	.96	1.25	4.13	Do.
51	7-8	79.30	20.70	45.12	217.97	152.66	31.60	39.86	152.66	1.42	1.70	do	.82	1.04	3.98	Do.
52	9-10	78.30	21.70	35.58	163.96	176.59	38.32	48.96	176.59	2.95	1.08	Negative	.69	.88	3.17	Do.
53	(?)	76.70	23.30	52.08	223.51	75.62	17.62	22.98	75.62	2.95	.34	Sucrose	1.06	1.38	4.54	Do.

¹ By refractometer.² Whole stalk.

TABLE 4.—Solids, sucrose, reducing sugars, and starch content of sirups made from various internodes of Hodo variety of sorgo at different stages of maturity

MILK STAGE

Sirup No.	Inter-nodes	Total solids ¹	Water ¹	True sucrose (Clerget)			Reducing sugars as invert sugar			Sugar ratios		Crystallization	Starch			
				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose		On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Jellying
54	Nos. 1-2	Percent 73.70	Percent 26.30	Percent 25.59	Grams 34.72	Grams 97.30	Percent 40.40	Grams 54.81	Grams 153.61	0.63	1.58	Dextrose----	Percent 0.43	Grams 0.59	Grams 1.65	Negative.
55	3-4	78.00	22.00	35.58	45.61	161.72	37.17	47.65	168.95	.95	1.04	do	.29	.38	1.34	Do.
56	5-6	73.30	26.70	28.61	39.03	107.15	40.50	55.24	151.68	.70	1.41	do	.17	.24	.66	Do.
57	7-8	72.00	28.00	21.80	30.27	77.85	47.25	65.62	163.75	.46	2.17	do	.16	.23	.59	Do.
58	9-10	71.50	28.50	14.38	20.11	50.45	54.95	76.84	192.80	.26	3.82	do	.15	.22	.55	Do.
59	11-12	71.80	28.20	11.96	16.66	42.41	56.60	78.82	200.70	.21	4.73	do	.07	.10	.25	Do.
60	13-14	72.20	27.80	8.33	11.54	29.96	60.15	83.30	216.36	.14	7.22	do	.04	.06	.14	Do.
61	(2)	72.10	27.90	21.50	29.82	77.06	46.70	64.76	167.38	.46	2.17	do	.13	.19	.49	Do.

DOUGH-TO-RIPE STAGE

Sirup No.	Inter-nodes	Total solids ¹	Water ¹	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose	Crystallization	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Jellying
62	1-2	78.20	21.80	46.78	59.83	214.58	25.15	32.17	115.36	1.86	0.54	Sucrose	0.52	0.67	2.40	Negative.
63	3-4	81.40	18.60	48.15	59.16	258.87	29.37	36.09	137.90	1.64	.61	do	.44	.55	2.40	Do.
64	5-6	77.70	22.30	39.36	50.66	176.50	34.20	44.02	153.36	1.15	1.87	do	.40	.52	1.81	Do.
65	7-8	77.60	22.40	32.85	42.34	146.05	40.77	52.55	182.00	.80	1.24	Dextrose	.26	.34	1.17	Do.
66	9-10	78.90	21.10	25.44	32.25	120.56	49.85	63.19	236.25	.51	1.96	do	.21	.27	1.01	Do.
67	11-12	72.60	27.40	16.96	23.36	61.89	51.35	70.74	187.40	.33	3.03	do	.13	.19	.50	Do.
68	13-14	73.80	26.20	16.19	21.94	61.79	54.95	74.47	209.73	.29	3.39	do	.11	.16	.45	Do.
69	(2)	75.00	25.00	36.49	48.66	145.96	34.22	45.63	136.88	1.06	.94	Negative	.32	.43	1.29	Do.

DEAD-RIPE STAGE

Sirup No.	Inter-nodes	Total solids ¹	Water ¹	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Sucrose to reducing	Reducing to sucrose	Crystallization	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water	Jellying
70	1-2	80.30	19.70	52.23	65.04	265.12	20.87	25.99	105.93	2.50	0.40	Sucrose	0.76	0.95	3.87	Negative.
71	3-4	81.30	18.70	53.44	65.72	285.77	24.02	29.54	128.44	2.22	.45	do	.54	.67	2.91	Do.
72	5-6	77.30	22.70	47.60	61.49	209.42	26.02	34.43	117.26	1.78	.56	do	.40	.64	2.17	Do.
73	7-8	77.40	22.60	42.60	55.15	188.89	31.92	41.23	141.23	1.33	.74	do	.31	.49	1.40	Do.
74	9-10	75.20	24.80	35.43	47.11	142.86	37.07	49.29	149.47	.96	1.04	Dextrose	.25	.33	1.00	Do.
75	11-12	75.10	24.90	33.16	44.15	133.17	38.97	51.88	156.50	.85	1.17	do	.20	.27	.81	Do.
76	13-14	76.00	24.00	35.28	46.41	147.00	37.65	49.53	156.87	.94	1.07	do	.18	.24	.76	Do.
77	(2)	75.30	24.70	39.36	52.26	159.35	33.05	43.89	133.80	1.19	.84	Sucrose	.42	.56	1.76	Do.

¹ By refractometer.² Whole stalk.

Sucrose crystallized from 21 sirups, dextrose crystallized from 45, and 26 jellied. Only 2 sirups neither crystallized nor jellied. Eleven of the 26 sirups that jellied crystallized sucrose, 6 crystallized dextrose, and 9 jellied without crystallization.

With the exception of sirups from the Honey variety at the two later stages of maturity, when this variety dropped its seed and branched prolifically (12), the different sirups showed the following general tendencies.

DEXTROSE CRYSTALLIZATION

Reducing sugar content and its ratio to sucrose content were higher in sirups made from less mature sorgo and, in general, in sirups made from the lower portions of the stalk at each stage of maturity.

Dextrose crystallization was correlated with the ratio of reducing sugars to sucrose, occurring in 45 sirups in which this ratio was greater than 1. It did not occur, however, in 4 sirups in which this ratio was greater than 1.

The number of portions of the stalk giving sirups that crystallized dextrose decreased with maturity.

Dextrose crystallization occurred in sirups from all four varieties, but the extent varied.

SUCROSE CRYSTALLIZATION

Sucrose content and its ratio to reducing sugars were higher in sirups made from more mature sorgo and, in general, from the upper portions of the stalk.

Sucrose crystallization was correlated with the ratio of sucrose to reducing sugars, occurring in 21 sirups in which this ratio was greater than 1. It did not occur, however, in 7 sirups in which the ratio was greater than 1.

The number of portions of the stalk giving sirups that had ratios of sucrose to reducing sugars greater than 1 and which crystallized sucrose increased with maturity.

Sucrose crystallization occurred in sirups from all four varieties, but the extent varied in sirups from the different varieties.

STARCH

In general, the starch content was highest in sirups made from the upper internodes, and decreased progressively in sirups made from internodes toward the bottom of the stalk. As a rule, both the number of sirups that jellied and their starch content increased with maturity of the sorgo.

Jellying was correlated largely with high starch content of the sirups, although it also depended on the content of starch per 100 gm. of water. The minimum amount of starch per 100 gm. of water in sirups in which jellying occurred was 2.25 gm. (in sirup No. 29).

High starch content and the resulting jellying appear to have inhibited sucrose crystallization in five sirups (Nos. 14, 15, 20, 28, and 41) and dextrose crystallization in four (Nos. 17, 35, 36, 52).

In tables 5 and 6 the sirups are regrouped according to their crystallizing and jellying characteristics.

TABLE 5.—*Sucrose and starch content of sirups grouped according to tendency to jelly and to crystallize sucrose*

SIRUPS THAT CRYSTALLIZED SUCROSE BUT DID NOT JELLY											
Sirup No.	Sucrose			Rates of sucrose to reducing sugars	Starch per 100 gm. of water	Sirup No.	Sucrose			Ratio of sucrose to reducing sugars	Starch per 100 gm. of water
	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water		
	Percent	Grams	Grams		Grams		Percent	Grams	Grams		Grams
8.....	39.97	50.40	193.09	1.22	1.91	70.....	52.23	65.04	265.12	2.50	3.87
45.....	41.79	53.99	184.91	1.32	2.19	71.....	53.44	65.72	285.77	2.22	2.91
62.....	46.78	59.83	214.58	1.86	2.40	72.....	47.54	61.49	209.42	1.78	2.17
63.....	48.15	59.16	258.87	1.64	2.40	73.....	42.69	55.15	188.89	1.33	1.40
64.....	39.36	50.66	176.50	1.15	1.81	77.....	39.36	52.26	159.35	1.19	1.76

SIRUPS THAT CRYSTALLIZED SUCROSE AND ALSO JELLIED											
16.....	38.30	49.74	166.52	1.20	3.34	49.....	55.41	69.29	277.05	3.02	5.20
29.....	40.58	52.49	178.76	1.27	2.25	50.....	49.21	64.10	212.11	2.19	4.13
42.....	52.38	63.95	289.39	2.43	4.66	51.....	45.12	56.92	217.97	1.42	3.98
43.....	51.32	65.53	236.49	2.26	3.46	53.....	52.08	67.93	223.51	2.95	4.54
44.....	47.24	60.63	213.76	1.74	2.32	47.....	46.33	59.77	205.91	1.76	2.96
48.....	47.99	67.62	165.48	3.21	3.98						

SIRUPS THAT HAD RATIOS OF SUCROSE TO REDUCING SUGARS GREATER THAN 1 BUT DID NOT CRYSTALLIZE SUCROSE

7.....	34.37	46.63	130.68	1.15	1.68	28.....	38.76	48.75	189.07	1.31	2.56
14.....	41.33	54.52	170.78	1.74	3.57	41.....	37.40	47.58	174.76	1.05	3.74
15.....	44.21	57.48	191.38	1.77	3.59	69.....	36.49	48.66	145.96	1.06	1.29
20.....	37.40	50.46	144.40	1.21	2.60						

TABLE 6.—*Sucrose and starch content of sirups grouped according to tendency to jelly and to crystallize dextrose*

SIRUPS THAT CRYSTALLIZED DEXTROSE BUT DID NOT JELLY											
Sirup No.	Reducing sugars as invert sugar			Ratio of reducing sugars to su- crose	Starch per 100 gm of water	Sirup No.	Reducing sugars as invert sugar			Ratio of reducing sugars to su- crose	Starch per 100 gm of water
	On sirup basis	Per 100 gm. of solids	Per 100 gm. of water				On sirup basis	Per 100 gm. of solids	Per 100 gm. of water		
	Percent	Grams	Grams		Grams		Percent	Grams	Grams		Grams
1.....	52.45	66.13	253.38	3.23	1.91	37.....	50.02	70.14	174.28	2.78	1.66
2.....	50.57	67.33	203.09	2.61	1.08	40.....	63.05	88.12	227.61	11.26	2.16
3.....	51.90	66.02	242.52	2.36	1.10	46.....	38.37	51.64	149.30	1.21	1.70
4.....	54.05	69.37	244.57	2.78	.88	54.....	40.40	54.81	153.61	1.58	1.65
5.....	65.95	86.20	280.63	11.46	.70	55.....	37.17	47.65	168.95	1.04	1.34
6.....	64.50	87.03	249.63	15.21	.27	56.....	40.50	55.24	151.68	1.41	.66
9.....	39.90	53.41	157.70	1.34	1.12	57.....	47.25	65.62	168.75	2.17	.59
10.....	48.24	66.90	172.90	2.39	.56	58.....	54.95	76.84	192.80	3.82	.55
11.....	56.11	72.12	240.81	3.37	.39	59.....	56.00	78.82	200.70	4.73	.25
12.....	56.63	72.32	260.96	3.43	.39	60.....	60.15	83.30	216.36	7.22	.14
13.....	36.80	48.74	150.20	1.10	.83	61.....	46.70	64.76	167.38	2.17	.49
18.....	43.49	58.53	170.54	1.46	1.69	65.....	40.77	52.55	182.00	1.24	1.17
19.....	40.81	56.13	149.49	1.64	1.54	66.....	49.85	63.19	236.25	1.96	1.01
24.....	57.55	78.33	217.17	4.42	1.77	67.....	51.35	70.74	187.40	3.03	.50
25.....	65.75	91.82	231.51	22.83	1.37	68.....	54.95	74.47	209.73	3.39	.45
26.....	65.30	88.96	245.49	48.01	.97	74.....	37.07	49.29	149.47	1.04	1.00
31.....	44.50	60.54	167.92	1.72	2.07	75.....	38.97	51.88	155.50	1.17	.81
32.....	54.95	71.82	233.83	3.21	2.37	76.....	37.65	49.53	156.87	1.07	.76
33.....	56.15	77.33	204.93	5.29	1.48	27.....	50.85	66.58	215.46	2.50	1.94
34.....	45.25	62.15	166.36	1.87	1.90						

SIRUPS THAT CRYSTALLIZED DEXTROSE AND ALSO JELLIED											
21.....	38.80	50.01	173.21	1.61	3.67	30.....	38.20	50.32	158.50	1.10	2.74
22.....	45.55	56.82	230.05	1.66	3.28	38.....	48.10	65.61	180.15	2.14	2.77
23.....	56.20	69.07	302.15	2.69	2.67	39.....	62.75	88.12	217.88	11.84	2.29

SIRUPS WITH RATIOS OF REDUCING SUGARS TO SUCROSE GREATER THAN 1 THAT DID NOT CRYSTALLIZE DEXTROSE BUT JELLIED

17.....	35.89	47.36	147.69	1.08	2.36	36.....	40.50	50.62	202.50	1.26	3.00
35.....	40.75	52.98	176.40	1.68	2.40	52.....	38.32	48.96	176.59	1.08	3.17

The sirups that crystallized sucrose but did not jelly are grouped in the first part of table 5. Sirup No. 77 had the lowest sucrose content per 100 gm. of water, 159.35, and sirup No. 64 had the lowest ratio of sucrose to reducing sugar, 1.15. In four sirups, Nos. 62, 63, 70, 71, the content of starch per 100 gm. of water was greater than 2.25 gm., which as previously noted was the minimum starch content of the sirups that jellied. High ratios of sucrose to reducing sugar and a relatively high content of sucrose per 100 gm. of water characterized these particular sirups.

The sirups that crystallized sucrose and likewise jellied are grouped in the second part of table 5. The minimum ratio of sucrose to reducing sugars in this group was 1.20, and the minimum content of sucrose per 100 gm. of water was 165.48, both of which are higher than the minimum values in part 1 of this table. The higher values for these sirups were probably due to the influence of jellying on crystallization.

Seven sirups with ratios of sucrose to reducing sugars greater than 1 which did not crystallize sucrose are listed in the last part of table 5. Five of these seven sirups jellied, which probably inhibited crystallization. Thirty-nine sirups that crystallized dextrose, but did not jelly are listed in the first part of table 6. The minimum content of dextrose per 100 gm. of water was 149.30 gm., with a minimum ratio of reducing sugar to sucrose of 1.04.

Data on six sirups that crystallized dextrose and also jellied are given in the second part of table 6. In these the proportion of reducing sugars per 100 gm. of water was greater than the minimum value, 149.30, in part 1. The ratios of reducing sugars to sucrose were also greater than the minimum value, 1.04, in the first part of this table. Probably the increase was due to the influence of jellying on dextrose crystallization.

The last part of table 6 presents data on four sirups with ratios of reducing sugar to sucrose greater than the minimum in part 1. All four of these sirups jellied, as will be noted by referring to tables 1 to 4, and three exhibited a greater content of reducing sugars per 100 gm. of water than the minimum in part 1. Here, again, the effect of jellying on crystallization is apparent.

CONCLUSIONS

From data obtained in a critical examination of 67 samples of sirup made from parts of the sorgo stalk and of 10 samples made from the whole stalk, the following conclusions are drawn.

The starch content and jellying of sorgo sirups are correlated and increase with maturity of the sorgo. The upper portions of the stalk produce sirups higher in starch content. The number of parts of the stalk yielding sirups that jelly increases with maturity.

Sucrose crystallization occurs most frequently in sirups made from the upper part of the sorgo stalk. The number of parts of the sorgo stalk yielding sirups from which sucrose crystallizes increases with maturity.

Dextrose crystallization occurs most frequently in sirups made from the lower portions of the stalk. The number of portions of the stalk yielding sirups from which dextrose crystallizes decreases with maturity.

Within the range of densities applying to farm-made sorgo sirups, either sucrose or dextrose may crystallize from sirups from different parts of the same stalk. In this study it was found that when the ratio of sucrose to reducing sugars in the sirup was 1.15 or greater sucrose crystallized and when the ratio of reducing sugars to sucrose was 1.04 or greater dextrose crystallized.

As a factor in the quality of sorgo sirup, crystallization of dextrose is as important as crystallization of sucrose.

By proper selection of the parts of the stalk for milling, either sucrose or dextrose may be obtained from the sorgo plant.

Jellying and either sucrose or dextrose crystallization may occur in the same sirup.

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THE PREPARATION AND PAINTING OF MAPLE-SUGAR-PRODUCING EQUIPMENT¹

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INTRODUCTION

Examinations by State and Federal authorities have disclosed the fact that maple sirup and other maple products offered in commerce sometimes contain a considerable quantity of lead. Occasionally the quantity is large enough to make the products unsafe for consumption. The maple sap as it comes from the tree has been proved by experiment to be lead-free. The lead must, therefore, be accumulated between the time the sap leaves the tree and its final processing. This limits the source of contamination to the lead-bearing surfaces of the processing equipment. These sources may be the lead-tin alloy (terneplate) coating used on some iron buckets, storage tanks, and evaporators; the lead-tin solder used in some of the joints of the metal equipment; or a paint, high in lead content, used to cover the tin-plated and wooden buckets and storage tanks to keep them from weathering.

Since all maple products produced in lead-free equipment are lead-free, a simple method of eliminating the danger of contamination would be to replace all equipment that has any lead-bearing surfaces. The cost of such a program would be prohibitive, however, and would force many maple producers out of business. A suitable material must, therefore, be found to cover up the old lead-bearing surfaces and to protect the tin-plated² and wooden buckets from weathering.

Such a covering may be any one of several types of paints, varnishes, lacquers, resins, etc., and the choice may be governed by many factors, the most important of which is the absence of lead. The coating should spread well when applied with a brush or as a spray. It should wear well and not chip, crack, blister, or peel when exposed to the action of sour sap, the temperature of boiling cleaning solutions, or mechanical shock unavoidable in the handling and storing of equipment.

Of the materials manufactured that might be suitable as a protective coating for maple-sirup-producing equipment, 20 were tested in the laboratory. Of these, only 8 were sufficiently low in lead (less than 100 parts per million) to be acceptable and so subject to further tests. It was found in these tests that the fact that a paint did not have its lead content printed on the label or indicated by the name was no proof that it did not contain lead. For example, 4 of the 6 aluminum paints submitted were discarded because they contained from 4 to 25 times too much lead.

¹ Received for publication December 1, 1938. Journal Paper No. 290 of the New York State Agricultural Experiment Station.

² Some of the tin-plated equipment has such a thin layer of tin that in a year or two it rusts through, and so must be protected.

The eight paints that passed the lead test were further examined in the laboratory and subjected as nearly as possible to all the conditions that would be encountered in the sugarbush. As a result of these tests, the paints were recommended to producers in the following order: (1) Kauri Grey Bakelite Resin Enamel, (2) Duco Aluminum, (3) Interior Tank Finish Aluminum, (4) S-W Bucket White Enamel, (5) du Pont Aluminum Paint 354-758, (6) G. L. F.³ Lead-free Aluminum, and (7) Plicote Laboratory Aluminum Paint. Since laboratory tests often fail to show the same results that would be obtained under actual working conditions, the first three paints named, those judged best in the laboratory, were tried out in the sugarbush. The other five were not given field trials because there was not time enough to make provisions for conducting additional trials.

MATERIALS AND METHODS

Since the paints were to be tested under actual operating conditions, the cooperating producers were told only how to prepare the buckets for painting and the temperatures at which to apply the paint. The method of application was left to them. Only buckets were painted in these trials because they are undoubtedly the greatest source of lead and because they were best adapted to the experimental work. It was deemed desirable to have the selection, preparation, and painting of the buckets done by the producers in their own way, so that the results obtained would be an indication of what might be expected when the paints recommended were in general use.

Since the length of a sap season is dependent entirely upon the weather, and since as wide a range of weather conditions as possible was desired for the field trials, the paints were tried in both St. Lawrence and Cortland Counties. The tests in St. Lawrence County were made to determine the effectiveness of the paint as a coating to prevent contact of the sap with the leaded surfaces. The effectiveness was measured by the amount of lead found in the sap throughout the season. The tests in Cortland County were made only to furnish additional information on the durability of the paint.

The producers cooperating in these experiments were selected because the sirup produced in their bushes during the 1937 season had been high in lead. The buckets used in the bush of one producer were all tin-plated and well covered with a white-lead paint. Those of another producer were all painted wooden buckets, and those of a third were painted wood, tin- and terne-plated buckets.

Each producer divided the buckets that were to be painted into two groups. The buckets of one group had only the loose paint brushed out and were then washed. Those of the other group had all the old paint removed, special care being taken to remove all of it from around the seams as well as from the side walls and bottom. To do this it was necessary to use a paint remover. The one recommended was a 10-percent, or stronger, solution of commercial trisodium phosphate; and it proved entirely satisfactory both from the standpoint of ease of handling and cost. The solution is most conveniently made by adding 1 pound of the powder to 1 gallon of water, either boiling or cold. This can be poured into the buckets cold and then heated or the boiling solution can be poured into the buckets. The best and easiest way, when a large number of buckets

³ Letters refer to Grange, Dairyman's League, and Farm Federation.

are to be cleaned, is to make up a sufficient volume of the phosphate solution in a large container, such as a scalding kettle, heat it to boiling, and dip each bucket in, then take it out and rinse. This concentration of trisodium phosphate is alkaline but not as dangerous to use as lye. The producers were warned to put their hands into the phosphate solution as little as possible and then to wash them with plenty of water. This solution when hot will remove old paint from buckets in from 2 to 15 minutes. After the paint is loosened, the phosphate solution is emptied out and the pail washed thoroughly with water to remove the last traces of the phosphate. If the phosphate is not completely removed, the new paint will blister.

The buckets were prepared for these experiments in February, and all the work of paint removal, washing, drying, and painting was done in a warm room. After the buckets were washed and rinsed, they were allowed to dry 3 to 7 days before they were painted. At least one of each type of bucket—tin, terne, and wood—from which the old paint had been entirely removed by the phosphate treatment, was painted with each of the test paints. All the buckets of the three types from which only the loose old paint had been removed were similarly treated. All of the newly painted buckets were cured for 3 weeks before they were used. At the beginning of the sap-running season, the trees were tapped by the producers and the buckets, as described in table 1, hung in each bush.

At the end of each run, a sample of sap was collected from each of the trial buckets, transferred to glass lead-free containers and taken to the laboratory. In the case of the buckets where the test paint was applied over the old paint, a composite sample was made up of aliquots taken from each of several buckets. More attention was given to the buckets in which the trial paint was applied over the old, for if satisfactory, this would be the easier way for a producer to paint his equipment.

When the sample was taken from a single bucket, 1 gallon of sap was used for analysis; when the sample was taken from several buckets, 2 gallons were used. The glass containers were made lead-free by washing first with strong alkali, then with strong nitric acid and rinsing with lead-free water. At the laboratory the sap was transferred to gallon enamel stockpots, which had likewise been treated to make them lead-free, and evaporated down to a volume that contained approximately 30 percent of sugar, which is about one-half the concentration of maple sirup. This gave a volume of nearly 250 cc. The sap was not evaporated to the density of sirup because the volume of the resulting solution would have been insufficient for making the analysis and for determining the exact moment at which to stop the evaporation when it reached a density of 32° Baumé. At no time was the sap filtered, for any cellulose filtering medium, cloth or paper, might absorb some of the lead.

When the sap had been sufficiently concentrated by evaporation, it was cooled to 20° C. and its specific gravity taken. From the specific gravity of each sample the weight of this dilute sirup which would give 10 gm. of 11-pound-per-gallon sirup was calculated. This calculated weight of the dilute sirup was used for the lead analysis. The method of analysis was a modification of the rapid colorimetric method of Perlman.⁴ The results, reported in parts per million, are

⁴ PERLMAN, J. L. RAPID COLORIMETRIC DETERMINATION OF LEAD IN MAPLE SIRUP. *Indus and Engin. Chem., Analyt. Ed.* 10: 134-135. 1938.

shown in table 1. The three maple sugarbushes in which these experiments were conducted were in locations sufficiently different to give a different number of runs of sap. After a sample was collected the bucket was emptied. The time at which the sap started to run again and the time at which the next sample was taken were recorded in order to find the length of time that the sap stood in the bucket. The daily temperature at 8 a. m., noon, and 8 p. m. were also recorded. These values are given in table 2.

TABLE 1.—Lead content of maple sap collected during 8 runs in sap buckets of different types having different surface coatings

Bucket type	Condi- tion of surface ¹	Paint used ²	Lead in run number—							
			1	2	3	4	5	6	7	8
Tin plate.....	Control..	Old paint left on..	P.p.m. 2.00	P.p.m. 3.38	P.p.m. 2.30	P.p.m. 2.55	P.p.m. 3.05	P.p.m. 3.10	P.p.m. 6.02	
	OPR.....	Kauri.....	.40	.38	.16	.10	.30	.52	.80	
	OP.....	do.....	.15	.40		.16	.82	2.05		2.58
	OPR.....	ITF.....	.23	.50	0	0	0	.25	.30	
	OP.....	do.....	.04	.40	.27	.80	1.20	1.60	.82	1.27
	OPR.....	DA.....	.12	.25		.45	.69	1.28	1.53	1.38
	OP.....	do.....	.28	.75	.33	.35	2.19	3.49	3.64	5.10
	Control..	Old paint left on..	1.67			1.86			5.50	12.80
	OPR.....	Kauri.....	0		0	.17		.57	.43	
	OP.....	do.....	0		0	.10		.32	.87	7.98
Terneplate ³ ..	Control..	Original surface..			.10	1.83		2.01	4.0	
	OPR.....	ITF.....	.22		0	0		.15	1.39	.55
	Control..	Old paint left on..	.22		.26	.30		.98	4.26	6.30
	OPR.....	Kauri.....	.13		.26	1.77			3.07	12.30
	OP.....	do.....	.14		.38	.27		1.00	.27	5.64
	OPR.....	ITF.....	.02		.23	.63		3.27	5.84	11.65
	OP.....	do.....			.36	.45		.83		
	OPR.....	DA.....			.22	.10	0	.38	1.77	
	OP.....	do.....			.07	.08	.41	.70	1.56	
	Control..	Old paint left on..								
Wood.....	OPR.....	do.....								
	OP.....	do.....								
	OPR.....	do.....								
	OP.....	do.....								
	OPR.....	do.....								
	OP.....	do.....								
	OPR.....	do.....								
	OP.....	do.....								
	OPR.....	do.....								
	OP.....	do.....								

¹ OPR=old paint entirely removed; OP=over old paint.

² Kauri=Grey Bakelite Enamel; ITF=Interior Tank Finish Aluminum; DA=Duco Aluminum.

³ Under terneplate there are 2 controls—(1) old paint, (2) original surface.

TABLE 2.—Time that sap stood in buckets per given run and its temperature during this period

Run No.	Time in buckets in—			Date	Temperature recorded			Temperature range for run		Mean temperature
	Bush A	Bush B	Bush C		8 a. m.	Noon	8 p. m.	Highest	Lowest	
	Hours	Hours	Hours		° F.	° F.	° F.	° F.	° F.	° F.
1	96		96	Mar. 22	35	60	38	60	38	40
2	45			23	54	62	67	67	32	45
3	24	8	4½	24	32	42				
4	36	24	24	25	36	38		38	36	37
				29	43	56	44	68	43	47.5
				30	50	68				
				31	54	52	56		20	
				Apr. 1	38	41	34	56		
				2	31	35				
5	168	168	168	4	35	20	28			37.5
				5	20	28	20			
				6	30	35	32			
				7	32	36	29			
				8	34					
				8		39	29	39	30	
6	48	48	48	9	32	30	29			37.5
				11	34					
7	48	48	48					50	35	
				12	43	45	50			44.5
				13	35	64				
8	132	96						48	45	
				14	58	72	64			
				15	53	50	50			54.5
				16	45	65	50			
				18	54	68				

¹ About 120 hours as ice and 40 hours as liquid.

EXPERIMENTAL RESULTS

A comparison of the values given in table 1 is shown in figure 1. The mean temperatures are given in table 2.

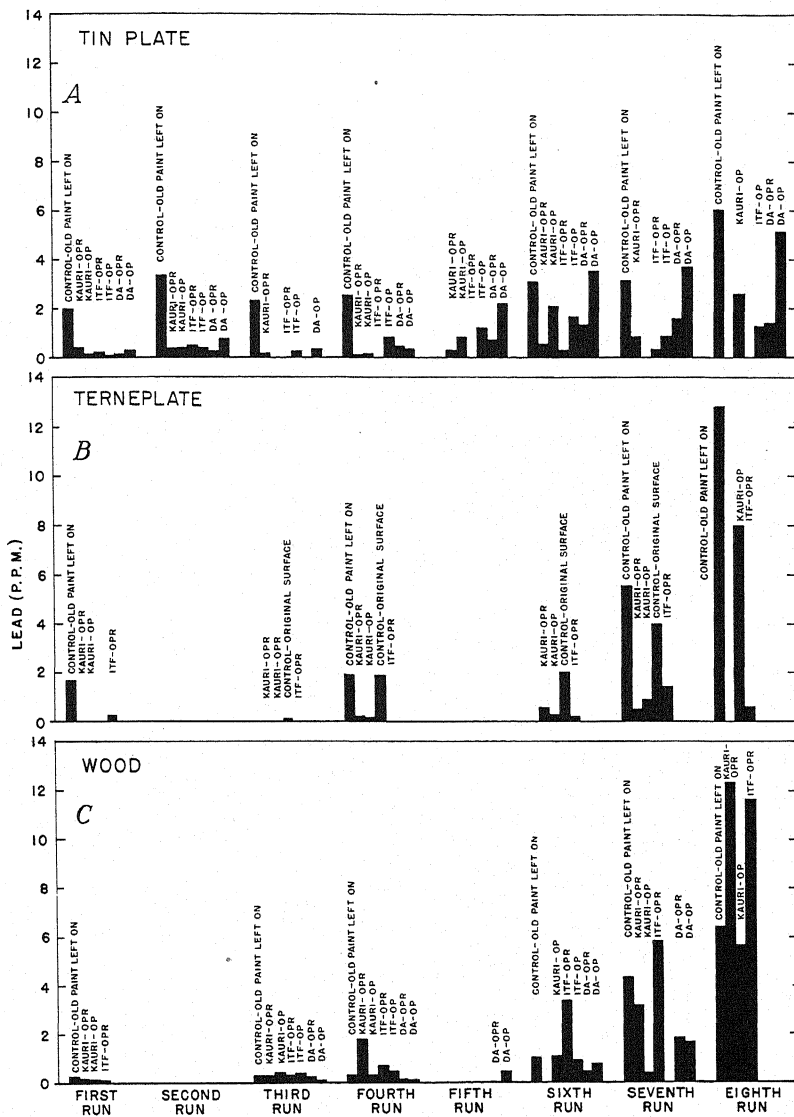


FIGURE 1.—Lead content of maple sap collected during eight successive runs in sap buckets of different types having different surface coatings: A, Tin-plated buckets; B, terneplate buckets; C, wooden buckets.

Comparing the lead dissolved by the sap in the control buckets where the sap was not protected from the leaded surfaces (fig. 1, A, B, C), the lead values are found to follow closely the mean temperature. This finding indicates that the lead-dissolving power of

the sap increases with increase in temperature; thus the higher the temperature the more lead dissolved. This no doubt is accounted for by the fact that the higher temperatures are optimum for the type of acid fermentation that occurs in the sap, and the amount of lead dissolved is directly proportional to the concentration of acid developed (the souring of the sap). During the first four runs of the sap the temperatures were low enough so that little or no fermentation took place. Consequently little acid was formed and the lead-dissolving power of the sap in all buckets was almost zero, the sap in the control buckets being nearly as low in lead as in the buckets that were protected by the trial paints. By the end of the fourth run the temperatures began to rise except during a freeze, that occurred in the middle of the fifth run. From that time on, the sap of the control buckets dissolved more lead on each successive run. The lead content of the sap, that was collected in buckets painted with the test paints remained almost constant throughout the season regardless of the test paint used, showing that the paints were about equally effective. The sap of the last run was allowed to stand in the buckets 132 and 96 hours. This is longer than a producer ever allows it to stand. Since the temperature at this time was high, the acidity of the sap was correspondingly high. This standing of the sour sap in the test buckets for so long a period subjected the paints to a severer test than they would have had under regular operating conditions. All three paints withstood this prolonged action of the warm, weak acid.

A comparison of the lead content of sap samples taken from the metal buckets, tin and terneplate, showed that the greatest protection afforded by the three paints was generally obtained when they were applied after the old paint has been entirely removed. Samples taken from the metal buckets where the test paints had been applied over the old paint contained from three to four times as much lead (fig. 1).

In the wooden buckets where the trial paints had been applied on surfaces from which the old paint had been entirely removed the amount of lead dissolved by the sap was much higher than in the buckets where the trial paints were applied over the old paint (fig. 1, C). This result, which is just the reverse of that obtained with the metal buckets, may have been due to the action of the paint remover on the wood fibers underlying the old paint.

All three of the paints when applied on wooden buckets, whether or not the old paint had been previously removed, withstood the effects of one sugar season very well. They neither chipped, blistered, nor peeled. This may have been due to the rigidity of the buckets and to the wood fiber surfaces upon which the paint was applied. The Grey Bakelite Resin Enamel at the end of the season showed some chipping on the bottom of some of the metal buckets, caused probably by the bulging of the bottoms during the freeze and the inability of the paint to follow the expansion of the metal because of its brittleness. No chipping of the bakelite enamel was shown in the earlier laboratory tests.

The two aluminum paints gave no evidence of chipping, blistering, or peeling from any of the buckets on which they were used, except in one bush where they were applied over old paint on metal buckets. There, toward the end of the season, a few showed blistering.

No one of the three paints had any effect upon the color, odor, or flavor of the sap. Each of the three producers expressed his preference for a different paint.

The producers cooperating in the Cortland County tests prepared and painted their buckets in a manner similar to that of the St. Lawrence County producers, but their buckets were not inspected until 1 month after the sugar season was over. The buckets had been stored away and so showed not only the effects of the use in the sugar-bush, but also that of handling at the time of storage. Fortunately, these buckets were stored by the nesting method, a method of storing that causes more abrasion than the stagger stack method. In these trials another paint, S-W Bucket White Enamel, was tried in addition to those that were tested in St. Lawrence County. No day-by-day record of temperature was kept. The season was extremely warm with only one hard freeze. This was excellent weather for testing the maple-sap bucket paints, the freeze being favorable to the chipping of the brittle paints, such as the two enamels, and the warm weather to the production of acid sap, which might cause blistering and subsequent peeling of the paint.

The results of these tests were as follows:

(1) The Bucket White Enamel was the hardest to apply, but when applied to wooden buckets it was entirely satisfactory. When applied to metal buckets, however, two out of every eight showed cracking and chipping. This paint gave no odor after 3 weeks of curing.

(2) The Kauri Grey Bakelite Resin Enamel was very easy to apply and gave much better coverage than the Bucket White. It stood up very well on the wooden buckets, but 1 out of every 10 of the metal buckets showed chipping. It still gave off a slight odor 3 weeks after painting.

(3) The Interior Tank Finish Aluminum stood up well on the wooden buckets, but when used on metal buckets 1 out of 12 showed peeling. This paint, like the Kauri, gave a slight odor after curing for 3 weeks.

(4) The Duco Aluminum was found by all the producers to be the easiest to apply. At the time of inspection, it was still entirely satisfactory, whether used on wooden or metal buckets. This paint, like the Bucket White Enamel, gave no odor after 3 weeks of curing.

The coverage, in number of buckets per quart of paint, was greatest for the aluminum paints. Next was the Kauri; the Bucket White Enamel gave the least satisfactory coverage. Eighty percent of the metal buckets in the Cortland County trials had never been painted before; where there was an old painted bucket of a given type used in the trials, there was a corresponding unpainted bucket. When there was chipping or peeling of the test paints, it occurred as often in the buckets in which the paint was applied over old paint as in those in which it was applied directly to the metal surfaces. This removes any question as to the chipping or peeling of the test paint being an aftereffect of the treatment by a paint remover. The producers noticed no color, flavor, or odor that might have been imparted to the sap or sirup by the test paint in any of these trials.

SUMMARY

The three paints, Kauri Grey Bakelite Resin Enamel, Interior Tank Finish Aluminum, and Duco Aluminum produce coatings that are very effective in keeping the lead of the leaded surfaces of sap buckets from being dissolved by the sap under normal conditions, the first two named being somewhat better in this respect than the Duco Aluminum. In commercial practice the great danger of excessive lead in maple sirup arises from the collection of sap under abnormal conditions, that is, where the sap has become very sour and is then allowed to stand in the buckets for a long time. Under these conditions the paints were, in a few cases, ineffective.

When painting metal buckets that have been painted before, the best results are obtained if the old paint is entirely removed.

A 10-percent solution of commercial trisodium phosphate, when used at or near the boiling temperature, is a very cheap and effective paint remover.

For wooden buckets that have been previously painted, better results are obtained if the new paint (any one of the approved ones), is applied over the old, any loose old paint being first removed by brushing.

If allowed to cure for at least 3 weeks after painting, none of the paints used in these experiments imparted any color, flavor, or odor to either the sap or the sirup.

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PHYTOPHTHORA TRUNK CANKER OR COLLAR ROT OF APPLE TREES¹

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INTRODUCTION

Trunk cankers have long been known to cause severe losses of trees in Indiana apple orchards. In some cases the damage has resulted from winter injury, or has been caused by fire blight (*Erwinia amylovora* (Burr.) Comm. S. A. B.). However, the cause of a particular type of destructive canker on the trunks of apple (*Malus sylvestris* Mill.) near the soil line, which occurs primarily on the Grimes Golden variety in Indiana, has not been definitely known. Following a serious epidemic of this type of collar rot in 1933, investigations of the nature of the disease and its control were undertaken. *Phytophthora cactorum* (L. and C.) Shroet. was found to be the causal agent. This paper presents the results of studies of the pathogenicity and physiologic specialization of the causal fungus, resistance and susceptibility of the host, and means of control of the disease.

THE DISEASE

HISTORY

Cankers caused by *Phytophthora cactorum* on the trunks of Grimes Golden trees, described by the writer (3, 5),³ are similar in many respects to collar rot cankers of undetermined or doubtful origin reported by several investigators. As early as 1858 the dying of apple trees as a result of an injury to the bark on the trunk and collar received considerable attention from fruit growers. According to Bradford and Cardinell (7), T. T. Lyon in addressing a meeting of horticulturists at Kalamazoo, Mich. in 1858, advocated the double-working of trees as a means of avoiding winter injury of the tender varieties. They mention that Baldwin, Tompkins King, Roxbury Russet, Rhode Island Greening, Esopus Spitzenburg, Hubbardston, and particularly Grimes Golden have long been listed as susceptible to collar injury. Stewart, Rolfs, and Hall (43), in 1900, described a collar rot of several varieties of apple trees in New York. They mentioned that the Tompkins King variety was so susceptible to attack that the disease was generally known as the king disease. Grossenbacher (17, 18), in 1909 and 1912, reported investigations on the nature of collar rot on apple trees in New York, and presented a review of the literature on this subject. He did not agree with Headden (19, 20) that arsenical poi-

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² The writer wishes to express his appreciation to Dr. R. M. Caldwell for helpful suggestions in the preparation of the manuscript and to Dr. Laurenz Greene and Prof. J. A. McClintock for their generous cooperation in making available most of the trees that were used for inoculation.

³ Italic numbers in parentheses refer to Literature Cited, p. 182.

soning was the cause of collar rot, but was of the opinion that much of the injury was induced by low winter temperatures. In 1926 Thomas (44) concluded that low winter temperatures caused a root and crown injury on apple trees in New York.

Collar rot of doubtful or unknown origin has been reported frequently. Selby (37), in 1900, in general attributed collar rot of apple trees in Ohio to winter injury. He mentioned that Grimes Golden, Tompkins King, and some other varieties, even in ordinary winters, seem to die on one side of the trunk just above the surface of the ground. Since the injury was not always confined to the south or southwest side of the trees, he expressed some doubt that it resulted entirely from the effects of winter temperatures. In an earlier bulletin (36) he reported that two orchards of 8- to 10-year-old Baldwin trees were ruined by winter freezing in 1881, while Grimes Golden trees under the same circumstances escaped. In 1913 Selby (38) stated that the very serious collar rot of Grimes Golden trees can be largely overcome by using top-grafted rather than root-grafted trees. Later, in 1921, Selby⁴ and in 1922 Thomas,⁵ reported a collar rot of undetermined origin on Grimes Golden and certain other varieties in Ohio. In reply to a question on collar rot, during a meeting in 1900 of the Indiana Horticultural Society, Burton (8, p. 66) stated:

It is quite likely that Mr. Tilson is troubled with what I term Grimes' bark disease. It is very much subject to bark disease, which affects it at the ground or a little above it. I don't know of any other tree so affected * * *

In 1921 Gardner (15) reported one orchard in southern Indiana in which approximately 25 percent of the Grimes Golden trees were affected with a collar rot, the cause of which was unknown. The disease appeared to be particularly serious on trees between 20 and 25 years of age. Anderson (1), in 1917, attributed a collar rot of Grimes Golden in Illinois to winter injury. However, the symptoms which he described are similar to those caused by *Phytophthora cactorum*. In 1918 Anderson (2) stated that collar rot, or Grimes Golden disease, appeared in orchards after the trees were about 10 years old, and frequently developed so rapidly that by the twentieth year, two-thirds of the Grimes trees were dead or in a dying condition. He suggested that double working or high grafting might offer a solution of the trouble. In 1919 collar rot of Grimes Golden trees in Illinois was adjudged to be a physiological condition, since attempts to isolate a causal organism failed (23). Anderson⁶ reported that in 1920 collar rot caused a crop loss of 1 percent in Illinois. Grimes Golden was the principal variety attacked. Anderson stated that the exact cause was unknown, but was probably winter injury.

Hotson (22), in 1920, concluded that most of the collar-rot cankers in the Yakima Valley, Wash., were due to fire blight, though the evidence that he presented was largely circumstantial. He listed a number of causes of collar rot, and stated that in the Yakima Valley comparatively few of the cankers can be traced to winter injury. As in many of the other publications reviewed, no mention was made of varieties affected. Magness (26), in 1929, concluded that low winter temperatures were the main cause of injuries occurring on the base of the trunks and roots of apple trees in Washington. He did not be-

⁴ ANDERSON, H. W. DISEASES OF FRUIT CROPS IN THE UNITED STATES IN 1920. U. S. Bur. Plant Indus., Plant Disease Bull. Sup. 14:1-114. 1921. [Mimeographed.]

⁵ HASKELL, R. J., and WOOD, JESSIE I. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1921. U. S. Bur. Plant Indus., Plant Disease Bull. Sup. 20:1-138. 1922. [Mimeographed.]

⁶ See footnote 4.

lieve that fire blight, as reported by Hotson (22), was an important contributing factor. A collar rot occurring on 15 varieties of apple trees in Pennsylvania was described by Orton and Adams (29) in 1915. They isolated the fire-blight organism from many of the cankers, and reported that typical cases of collar blight resulted from artificial inoculations into the collar and roots of young apple trees. The Grimes Golden, Baldwin, and York Imperial were listed as very susceptible. Many of the symptoms described were similar to those produced by *Phytophthora cactorum*. Orton and Adams found that trees between 7 and 20 years of age were affected, and that the average age of the trees when first infected was between 12 and 13 years, and that on many of the trees the canker was confined to the bark at the base of the trunk, and showed little advancement in a vertical direction.

Phytophthora cactorum causes cankers on the trunks and branches of many kinds of fruit and nut trees. Osterwalder (30), in 1912, reported the dying of young grafted apple trees in Switzerland, as a result of a killing of the bark near the graft union by *P. omnivora* de Bary, now considered synonymous with *P. cactorum*. Smith and Smith (42), in 1925, described a trunk and crown canker caused by *P. cactorum* and *P. citrophthora* (Smith and Smith) Leonian, on nursery and orchard trees of almond, apricot, cherry, peach, pear, plum, prune, and black walnut in California. A crown and trunk canker of walnut trees in California, caused by *P. cactorum*, was described by Smith and Barrett (40, 41) in 1930. Petri (31), in 1932, observed mycelium of a *Phytophthora* in the necrotic tissues of apple trees having collar rot, in Italy. In 1934 Curzi (10) described a crown rot of peach trees in Italy from which *P. cactorum* was isolated. Dunegan (12), in 1935, described a serious canker disease on the stems of young peach seedlings in Arkansas which was caused by *P. cactorum*. In 1938, Lindquist (25) described a collar rot of 10-year-old apple trees in Argentina from which *P. cactorum* was isolated. Smith (39), in 1937, reported infection on trees and shrubs of 26 plant genera artificially inoculated with a culture of *P. cactorum*. Tucker (46) extensively reviewed the literature and showed that *P. cactorum* is rather generally distributed throughout the temperate zones and parasitizes many different hosts. Tucker (45) and Smith (39) found that cultures of *P. cactorum* were omnivorous.

Tucker (46) has adequately reviewed much of the literature dealing with rots of apple and pear fruit caused by *Phytophthora cactorum*. Rose and Lindegren (34) obtained infection on uninjured pear and apple fruits placed in dishes containing orchard soil which was covered with water. Cooper (9) found that infection of uninjured apple and pear fruits by *P. cactorum* occurred through lenticels, and that zoospores may cause infection. Gardner (16) mentioned that apple fruit rot caused by *P. cactorum* occurred year after year in an orchard at Lafayette, Ind., and that the fungus apparently persists in the soil under the trees. Baines (4, 5) showed that *P. cactorum*, causing fruit rot, may also cause collar rot, and suggested that the growth of the fungus in the fruit may result in its increase in the soil.

OCCURRENCE OF PHYTOPHTHORA COLLAR ROT IN INDIANA ORCHARDS

During 1933 and 1934, years of severe collar rot epidemics in Indiana, apple trees in seven commercial orchards were examined for collar rot infection with respect to varieties and ages of the trees attacked.

In an orchard at Bedford, and in orchards designated as Nos. 1, 3, and 4 at Vincennes (table 1), an excellent opportunity was afforded

TABLE 1.—Occurrence of *Phytophthora* trunk cankers on Grimes Golden in 7 Indiana orchards, in 1933 and 1934

Location	Age of tree	Trees healthy	Trees affected		
			Less than ½ girdled	½ to completely girdled	Percentage
Bedford ¹	Years	Number	Number	Number	
	13	322	3	23	7.0
	18	28	16	26	60.0
Vincennes:					
Orchard No. 1 ²	14	58	4	54	50.0
	9	300	1	0	.3
Orchard No. 2.....	14	141	16	90	43.0
	19	216	5	11	7.0
Orchard No. 3 ³	11	277	2	1	1.0
	18	49	8	94	68.0
Orchard No. 4 ⁴	14	370	175	200	50.0
Bloomfield.....	16	370	5	92	21.0
Evansville.....	13	33	0	37	53.0

¹ No infection occurred in this orchard on 58 Delicious and 70 Winesap trees (18 years old), or on 86 Arkansas, 37 Baldwin, 388 Ben Davis, 185 Jonathan, 87 Maiden Blush, 173 Rome Beauty, 46 Stark, 171 Stayman Winesap, 18 Northern Spy, 25 Tompkins King, 8 Willowtwig, and 417 Winesap trees, which were 24 years old.

² No infection occurred in this orchard on 1,256 trees 14 years old, of the varieties Jonathan, Oldenburg, Rome Beauty, Stayman Winesap, Yellow Transparent, and York Imperial.

³ In this orchard of approximately 3,000 11-, 14-, and 18-year-old trees of the varieties Delicious, Grimes Golden, Jonathan, Oldenburg, Rome Beauty, Stayman Winesap, Yellow Transparent, Winesap, and York Imperial, the disease occurred only on Grimes Golden.

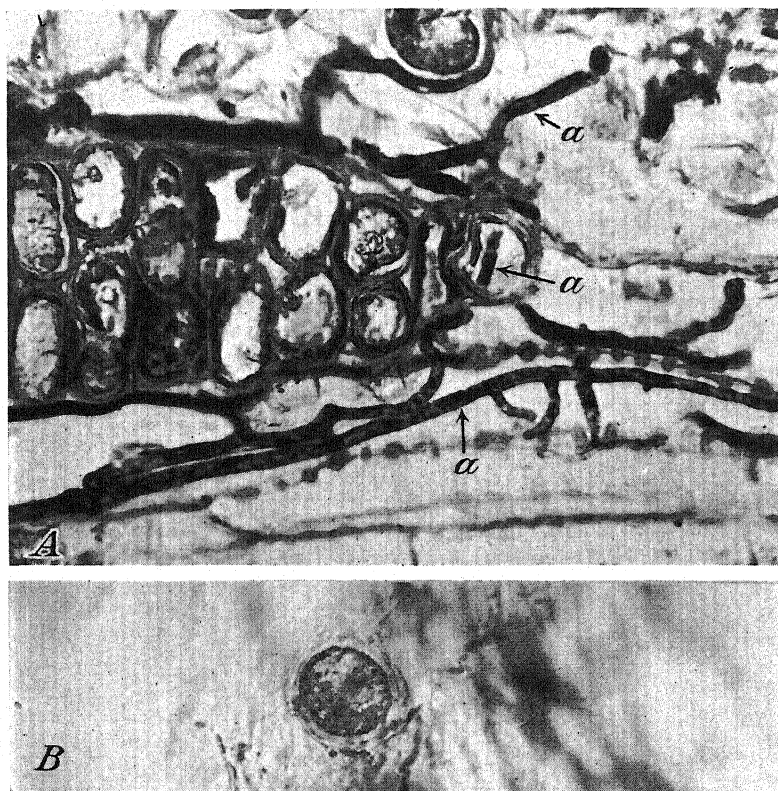
⁴ In this orchard, comprised of 220 acres of bearing trees of the varieties Arkansas, Ben Davis, Delicious, Golden Delicious, Grimes Golden, Jonathan, Maiden Blush, Oldenburg, Rome Beauty, Stayman Winesap, Yellow Transparent, Turley, Winesap, Winter Banana, Willowtwig, and York Imperial, infection, other than on Grimes Golden, occurred only on Rome Beauty, and only a few trees of this variety were infected.

to obtain information on the varieties affected, since in many cases other varieties were interplanted among the infected Grimes Golden trees. In these orchards, although Grimes Golden was severely attacked, no collar-rot cankers of a parasitic nature were observed on trees of the following varieties: Arkansas, Baldwin, Ben Davis, Delicious, Golden Delicious, Jonathan, Maiden Blush, Northern Spy, Oldenburg, Stark, Stayman Winesap, Tompkins King, Willowtwig, Winesap, Winter Banana, Yellow Transparent, and York Imperial. Two Rome Beauty trees affected with cankers which girdled the trunks were found in the orchard designated as No. 4 at Vincennes. The cankers appeared similar to those on Grimes Golden.

In six plantings of Grimes Golden trees, 14 to 18 years old, from 21 to 68 percent of the trees were infected with collar rot (table 1). In one planting of 19-year-old Grimes Golden trees 7 percent were infected. Of two plantings of 13-year-old Grimes Golden trees, one was 7 and the other 53 percent infected. Much less infection, 0.3 and 1 percent, occurred on plantings of Grimes Golden trees 9 and 11 years old, respectively. A discussion of this is presented later in connection with the report of inoculation experiments.

SYMPTOMS

Phytophthora cankers occur mainly on the trunks of the trees, but in later stages may involve the bases of the scaffold branches (fig. 1, C and D). Occasionally, roots near the surface of the ground are invaded. The trunk cankers, frequently irregular in shape, enlarge rapidly in both lateral and vertical directions and may girdle Grimes Golden trees within one season. On double- and high-grafted Grimes



A, Tangential section of infected apple bark showing (a) the intercellular and intracellular mycelium of *Phytophthora cactorum*; B, oospore of *P. cactorum* from infected apple bark. $\times 640$.

Golden, the cankers usually do not extend below the graft union, and consequently may not extend to the soil line (fig. 1, C). The first symptom of the disease is a wet, discolored area on the surface of the bark, resulting from an exudation of liquid from the killed bark (fig. 1, A, c). Frequently the cankers are well advanced, and the trunks may be completely girdled before any symptoms are noticeable to a casual observer. In later stages of infection, the bark becomes dry and the cankers are delimited by a definite margin. The bark on old cankers becomes cracked, and may pull away from the trunk (fig. 1, D). On severely infected trees the fruit ripens early, the foliage in the fall becomes a reddish-bronze color, and premature defoliation occurs (fig. 2, A). The affected trees blossom and leaf out the following spring, after which the leaves and fruit on branches directly above the girdled areas on the trunks usually wilt, and the branches die.

The affected bark of active cankers appears brown and water-soaked, and has a strong, fermented odor. Recently infected bark near the margins of enlarging cankers is light brown in color, with a gradual diminution of the brown color toward the healthy tissues (fig. 1, B, a). Occasionally, streaks extending 1 to 2 inches beyond the margin of the canker are found near the cambium. Usually the cambium is killed and the sapwood invaded and discolored. The enlargement of the cankers is checked in the fall, presumably by the maturation of the bark tissues. However, cankers which do not girdle trees in one season usually renew activity the following spring at various places on their margins.

PATHOLOGICAL HISTOLOGY

Infected bark from the margins of actively enlarging cankers on the trunks of 13-year-old Grimes Golden trees was fixed, infiltrated with paraffin, and sectioned by the usual method for histological examination. In sections stained in haematoxylin, and aniline blue in picric acid, the mycelium of the fungus, which stained blue, was readily differentiated from the host tissues. An abundance of both intercellular and intracellular coenocytic mycelium was observed in all parts of the infected bark (pl. 1, A, a). The infection caused a distinct disorganization of the host-cell protoplast and a break-down of the cell walls, especially of the parenchyma. Mycelium was not observed in advance of the discolored area of affected tissues.

Numerous oospores or chlamydospores, apparently those of *Phytophthora cactorum*, were found in mascerated bark collected in the spring from an overwintering canker produced by artificial inoculation (pl. 1, B). No sporangial stage of the fungus on the surface of the cankers was found by macroscopic examination of many cankers.

INFECTIOUS NATURE OF CANKER TISSUE

Early in the investigations, this type of collar rot was demonstrated to be infectious when cankers resulted from inoculations made by inserting pieces of infected bark into the trunks of five healthy Grimes Golden trees 13 years old. Inoculations were made with bark taken from each of five cankers. The inoculations were covered with cheesecloth and grafting wax. Typical cankers, from 5 to 15 cm. in diameter, resulted after 2 weeks from inoculations made with bark from four cankers.

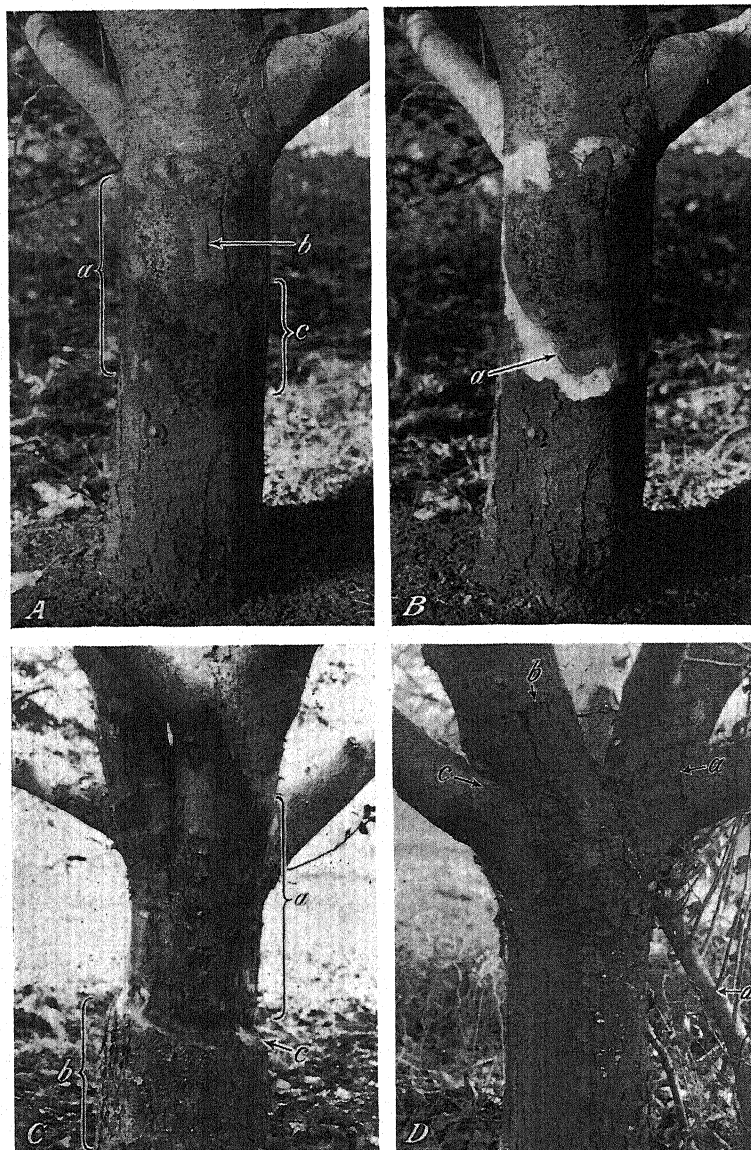


FIGURE 1.—*Phytophthora cactorum* cankers on Grimes Golden trees: A, Canker (a) on an 11-year-old tree 2 months after inoculation at b. The moist dark-colored area (c) is the first exterior symptom of the disease. B, The same canker as in A with the outer bark removed at a to show the light-brown, advancing margin of the canker. C, Trunk of tree illustrated in Figure 2, A. The canker (a) was confined to the trunk and bases of the main branches and did not invade the stock (b). The graft union (c) sharply limited the canker to the Grimes Golden tissues. Healthy light-colored tissue at the margin of the canker has been exposed by removal of the outer bark. D, Trunk of a 14-year-old tree girdled by an old canker with infected bark dry, cracked, and invaded by other organisms. The canker did not advance appreciably above the bases of the main branches at a, b, and c, nor infect the inarch (d) at the side of the trunk.

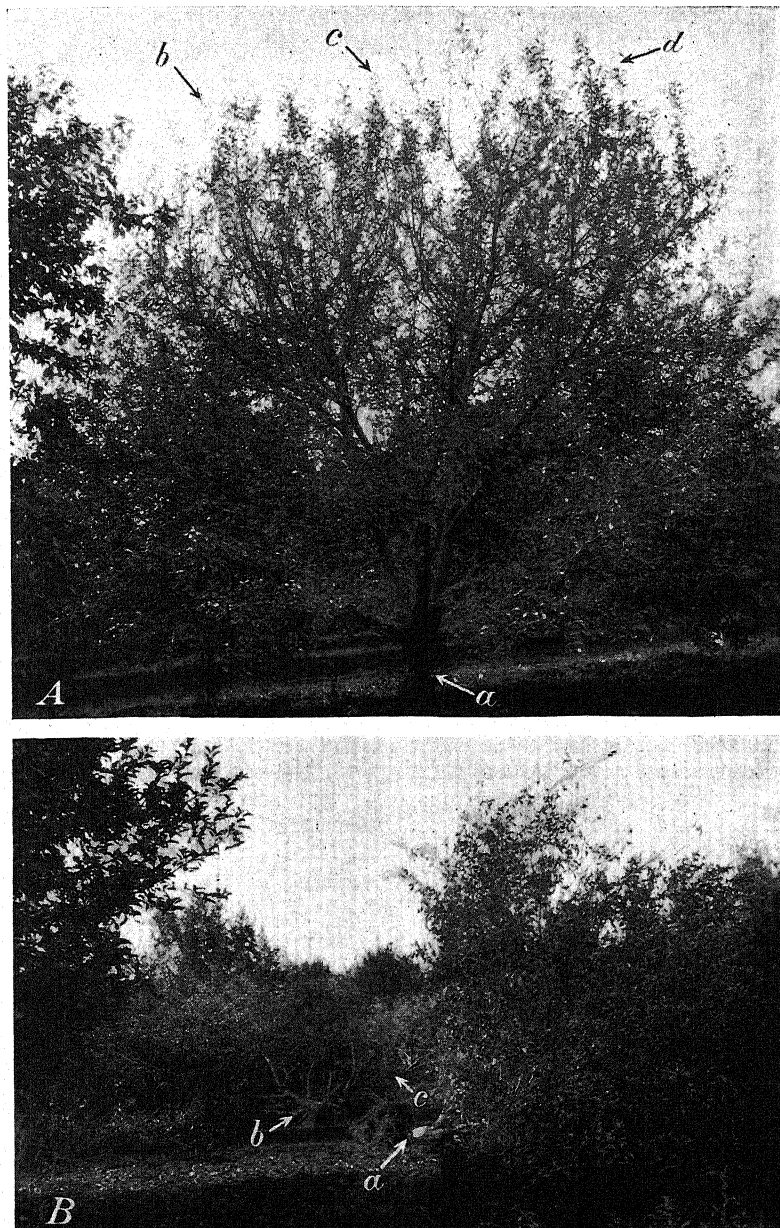


FIGURE 2.—*A*, 14-year-old, high-grafted Grimes Golden tree with trunk above the graft union (*a*) girdled by *Phytophthora cactorum* (see fig. 1, *C*). The tree was distinguished by the yellowish-green foliage, becoming reddish at the tips of the upper branches (*b*, *c*, and *d*), and by the partial defoliation. *B*, 14-year-old Grimes Golden trees (*a*, *b*, and *c*) girdled by *P. cactorum* and pulled up. The roots of 50 similar trees examined, except 1, were healthy.

RELATION OF BACTERIA TO THE DISEASE

Numerous bacterial colonies were obtained from infected bark of 17 trunk cankers of Grimes Golden trees by the dilution-plate method. Bacteria from these cankers were nonpathogenic when inoculated into 14- and 18-year-old Grimes Golden tree trunks, Briarcliff and Premier rose shoots, Rome Beauty and Winesap apple shoots, and Bartlett pear fruits.

Although pathogenic bacteria were not isolated from trunk cankers of Grimes Golden, inoculations were made on this variety with the fire blight organism, *Erwinia amylovora*, in an attempt to produce cankers typical of the collar rot disease. Three cankers less than 3 cm. in diameter, and delimited by cork at their margins, resulted from 12 inoculations made on the trunks of 11 14-year-old and 1 18-year-old Grimes Golden trees on May 11, 1934. Five bark cankers similar to these were also formed around the bases of inoculated succulent shoots which were on the trunks or bases of the first main branches of these trees. The culture of *E. amylovora* used in the inoculations was pathogenic on apple shoots and pear fruits. Obviously the trunk canker disease of Grimes Golden differed greatly from that caused by fire blight.

THE CAUSAL FUNGUS (PHYTOPHTHORA CACTORUM)

ISOLATION

*Phytophthora cactorum*⁷ was consistently isolated from small pieces of infected bark taken from the margins of enlarging cankers. The infected bark tissue was surface-sterilized in a solution of 1:1,000 mercuric chloride, twice rinsed in sterile water, and then plated on potato-dextrose agar. Other fungi, including an undetermined species of *Alternaria*, and a few bacteria, grew from some of the tissue plantings. No infection was obtained when the alternaria fungus was inoculated into the bark of the trunk of an 18-year-old Grimes Golden tree. The bacteria which grew from some of the plantings were not typical of *Erwinia amylovora*, and apparently were similar to those obtained by the dilution-plate method mentioned earlier.

During 1934-36, *Phytophthora cactorum* was obtained from 49 of the 64 cankers on the trunks of Grimes Golden trees from which isolation was attempted. The cankers from which the fungus was isolated were collected in 11 orchards in the vicinity of Bedford, Bloomfield, Evansville, Indianapolis, Mitchell, and Vincennes, Ind. All colonies of *P. cactorum* obtained from the 49 cankers, except 1, appeared to be similar in cultural characters on potato 2-percent dextrose agar. From 1 canker both an atypical culture and a typical culture of *P. cactorum* were isolated.

The isolation of *Phytophthora cactorum* from inactive cankers was found difficult by the tissue-planting procedure. However, when apple fruits were inoculated with small pieces of infected bark from the margins of such cankers, a rot frequently developed from which *P. cactorum* was readily isolated. The percentage of inoculations of this type producing infection of the fruits was rather low when the bark was secured from the centers of cankers.

⁷ The writer gratefully acknowledges the assistance of Dr. C. M. Tucker in the identification of the culture.

Fallen apples on the ground were frequently found rotted by *Phytophthora cactorum* during 1934-36. Cultures of *P. cactorum*, which appeared similar to those obtained from cankers, were obtained from 46 Grimes Golden, 5 Baldwin, 2 Stayman Winesap, and 3 Thompkins King fruits.

Phytophthora cactorum was also isolated from orchard soil. Twenty-eight samples of surface soil were collected in five orchards on October 15, 1935, and 7 and 11 samples were collected in an orchard at Lafayette on September 29 and October 24, 1936, respectively. The soil from each sample was introduced into shallow holes made in Grimes Golden fruits that had been picked at a height of 4 to 6 feet and washed in 95-percent alcohol. The soil was sealed in with petroleum jelly, and the inoculated apples were placed in waxed paper bags to retard drying, and incubated at 24° C. After 9 days decay was evident around many of the inoculations, and tissue plantings were made from the rots more or less typical of *P. cactorum*.

No cultures of *Phytophthora cactorum* were isolated from the fruits inoculated with soil collected on October 15, 1935. Cultures were obtained from 2 of the 7 samples of soil collected on September 29, 1936, and from 1 of the 11 samples collected on October 24, 1936. From many of the inoculations a soft rot developed which was not typical of *P. cactorum*. No decay developed on 12 fruits which were punctured with a cork borer, nor on 4 into which sterile soil was introduced.

PATHOGENICITY

MATERIAL AND METHODS

To test the pathogenicity of *Phytophthora cactorum* on Grimes Golden trees, 12 cultures were selected as being representative of the cultures in type of growth on potato-dextrose agar. Of the cultures selected, Nos. 1 to 7 (table 2) were isolated from cankers on the trunks of Grimes Golden trees at Bedford, Evansville, and Vincennes; Nos. 9 to 11 from apple fruits at Lafayette and Vincennes; and Nos. 26 and 27 from soil at Lafayette. The pathogenicity of culture 1 also was tested on trees of two additional species of apple, on two species of cherry, on three of plum, and on one of peach, pear, and quince which were growing in a field plot.

Inoculations were made with these cultures on 140 Grimes Golden apple trees, 2 to 30 years old, during 1934-38. All the trees inoculated, unless otherwise stated, were growing normally. Nearly all the inoculations were made during June, July, and August. The trees were inoculated by inserting a small quantity of mycelium and agar into a deep incision made with the point of a scalpel. The inoculum was obtained from colonies 3 to 5 days old grown on potato 2-percent dextrose agar. The inoculations were covered with cheesecloth and sealed with warm, low melting point grafting wax (fig. 3, A). Before each inoculation the instruments used were flamed. During 1934 the check incisions were made similarly to the above, except that no inoculum was introduced into the wound. However, in 1935-36 a small quantity of sterile potato-dextrose agar was inserted in the check incisions.

RESULTS OF INOCULATIONS

All the cultures except No. 2 (table 2) were severely pathogenic on the trunks of Grimes Golden trees between 8 and 30 years of age

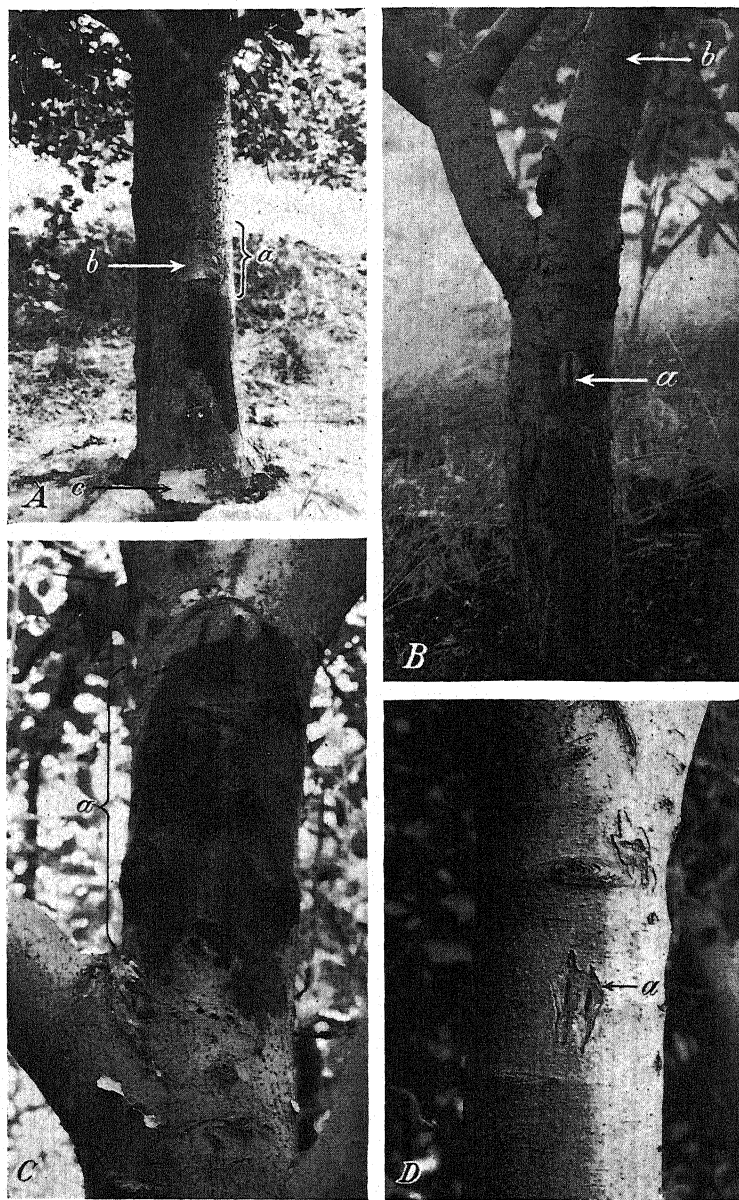


FIGURE 3.—A, Infection (a) not externally visible on trunk of a 13-year-old Grimes Golden tree 3 weeks after inoculation at (b). The wet, discolored area below the canker caused by exudation of a liquid from the infected area is the first external symptom of the disease. No infection resulted from an inoculation (c) on the Virginia crab stock. B, Inoculation incision (a) on a resistant (Jonathan) variety, with outer bark cut away to show the sharp limitation of the disease; check incision (b) on branch, which was similar to that on the trunk. C, Canker (a) produced by artificial inoculation on Grimes Golden branch 15 cm. in diameter. D, Slight enlargement at (a) of an inoculation incision, which had ceased enlarging and was classified as infection failure, on a Grimes Golden branch 8 cm. in diameter.

Cultures Nos. 1, 3 to 7, 9 to 11, 26, and 27 were severely pathogenic on trees 8 or more years old. Usually the cankers were 20 to 25 cm. in diameter 1 month after inoculation, and showed no evidence of being checked (fig. 1, A, B). In many cases the cankers girdled the 11- to 14-year-old Grimes trees within 3 months after inoculation. *Phytophthora cactorum* was obtained from each of the 32 cankers from which reisolation was attempted. No infection occurred on the 2- to 4-year-old trees, which were inoculated with culture No. 1.

A canker resulted from only one of five inoculations made with culture No. 2, although it was isolated from a canker and was similar in culture to the highly parasitic strains. This canker increased rapidly in size, suggesting that with this culture infection is established with difficulty, although it is capable of developing once established.

TABLE 2.—*Pathogenicity of Phytophthora cactorum on Grimes Golden trees in Indiana orchards*

Source of culture	Culture No.	Year of inoculation	Age of trees	Trees inoculated	Inoculations ¹	
					Total	Producing cankers ²
			Years	Number	Number	Number
Canker.....	1	1935	2	5	8	0
Do.....	1	1936	3	5	8	0
Do.....	1	1937	4	5	8	0
Do.....	1	1938	5	3	6	1
Do.....	1	1935	8	3	3	2
Do.....	1	1934	11	1	2	3
Do.....	1	1936	11	12	15	13
Do.....	1	1935	12	4	4	4
Do.....	1	1934	13	12	14	13
Do.....	1	1935	14	33	33	25
Do.....	1	1934	18	2	8	8
Do.....	1	1935	19	6	13	12
Do.....	1	1935	20	28	29	27
Do.....	1	1935	30	2	6	5
Do.....	2	1935	19	2	5	1
Do.....	3	1936	11	1	2	2
Do.....	3	1935	19	2	5	5
Do.....	4	1935	19	2	5	5
Do.....	5	1935	19	2	5	5
Do.....	6	1935	19	2	5	4
Do.....	7	1935	19	2	5	5
Fruit rot.....	9	1935	19	2	5	4
Do.....	10	1936	11	1	2	4
Do.....	11	1935	19	2	5	4
Soil.....	26	1936	11	2	4	3
Do.....	27	1936	11	2	4	2

¹ No cankers resulted from the 113 check incisions.

² *P. cactorum* was obtained from each of 32 cankers from which reisolation was attempted.

The results of inoculation of the trunks of species of *Prunus*, *Pyrus*, and *Cydonia* with culture No. 1 of *Phytophthora cactorum* are presented in table 3. Two-year-old trees of *Prunus mahaleb* L. (cherry), *P. avium* L. (cherry), *P. americana* Marsh. (plum), and 6-year-old trees of *P. persica* (L.) Batsch. var. Elberta (peach), and 1 tree of *P. domestica* L. var. Reine Claude (plum), 20 years or more old, were highly susceptible to infection by *P. cactorum* (fig. 4, A, B, C). Only one of five 2-year-old trees of *Prunus cerasifera* Ehrh. (plum) became infected. No infection occurred on 2-year-old trees of *Pyrus baccata* L. (apple), *P. coronaria* L. (apple), *P. serotina* Rehd. (pear), and *Cydonia oblonga* Mill. (quince).

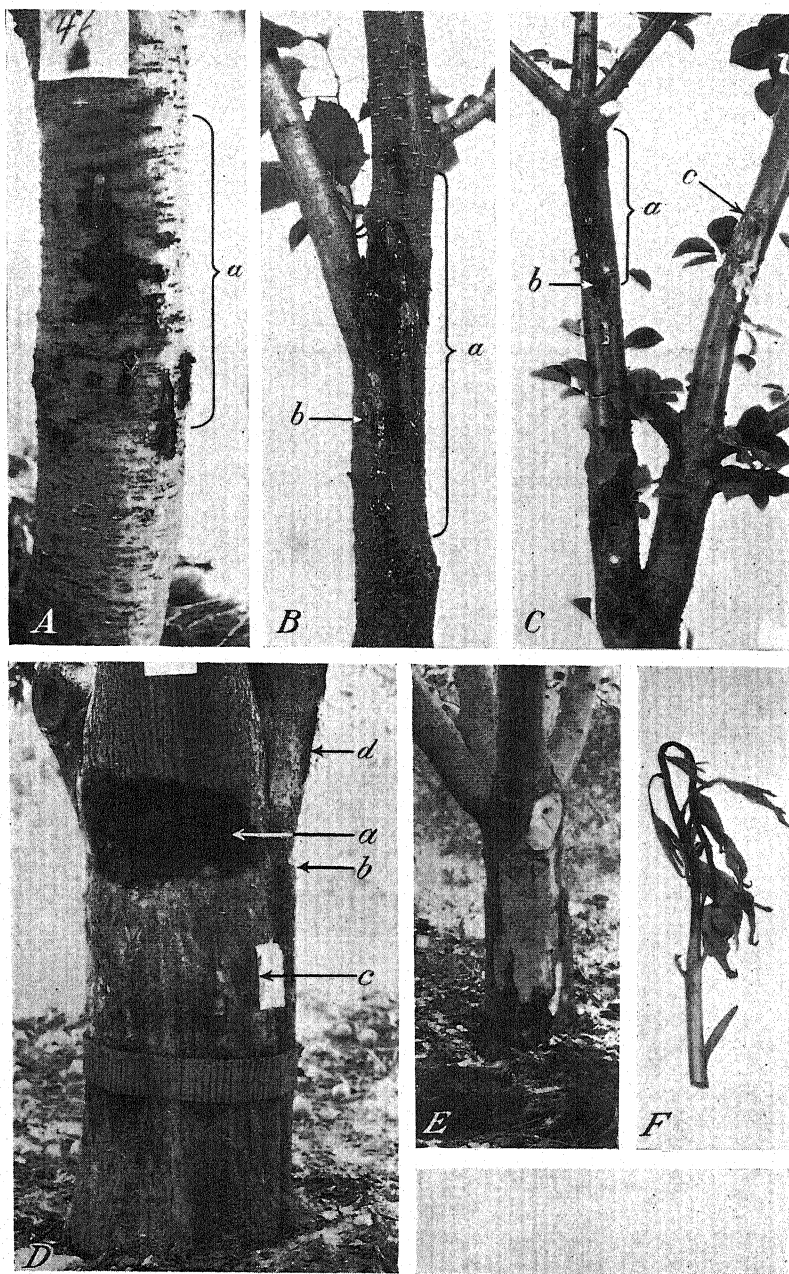


FIGURE 4.—A, Canker (a) on 6-year-old peach tree (artificial inoculation). B, Canker (a) on 2-year-old mazzard cherry tree resulting from inoculation at b. C, Canker (a) on 2-year-old mahaleb cherry tree resulting from inoculation at b; check incision at c. D, Inoculations on 20-year-old, double-worked Grimes Golden tree producing canker (a) on Grimes scion, which extended to graft union (b), but no infection from inoculation on Delicious stock (c), nor from check incision (d) on Grimes scion. E, Canker on trunk of 14-year-old Grimes Golden tree treated by scarification and ready for painting with Bordeaux paint. F, Infection of branch ready for painting with Bordeaux paint.

TABLE 3—Results of inoculation of trees of *Prunus*, *Pyrus*, and *Cydonia* species with culture No. 1 of *Phytophthora cactorum*

Species	Age of tree	Trunks inoculated	Cankers produced ¹	Check incisions ²
		Number	Number	Number
<i>Prunus avium</i>	2	5	3	5
<i>Prunus mahaleb</i>	2	5	5	5
<i>Prunus americana</i>	2	5	4	5
<i>Prunus cerasifera</i>	2	5	1	5
<i>Prunus domestica</i>	20	3	2	0
<i>Prunus persica</i>	6	3	3	3
<i>Pyrus baccata</i>	2	5	0	5
<i>Pyrus coronaria</i>	2	5	0	5
<i>Pyrus serotina</i>	2	5	0	5
<i>Cydonia oblonga</i>	2	5	0	5

¹ Apparently pure cultures of *Phytophthora cactorum* were reisolated from cankers on *Prunus avium*, *P. mahaleb*, *P. americana*, and *P. domestica*.

² No infection resulted from the check incisions.

³ Inoculations made on large branches.

No infection resulted from the check incisions during these tests. This fact was considered to justify the practice of not sterilizing the surface of the bark prior to inoculating. The orchards in which the inoculations were made were relatively free from fire-blight and collar-rot infection.

PHYSIOLOGIC SPECIALIZATION

MATERIAL AND METHODS

The inoculations to differentiate physiologic races of the fungus, unless otherwise stated, were made in a 10-year-old orchard of Grimes Golden trees, which contained 1 or 2 trees of each of the 30 other varieties listed in table 5 and footnote 2 of that table. These trees appeared normal, and a week before they were inoculated in July 1936, between 300 and 400 gallons of water were applied under each tree to insure plentiful soil moisture during the experiment. The sources of the 12 cultures of *Phytophthora cactorum* tested are given in table 4. Inoculations were made on the trunk of each tree, except Grimes Golden, with the 10 cultures Nos. 1, 8, and 12 to 19. Each of the 12 cultures was tested on one or more Grimes Golden trees. One check incision on each tree, into which sterile potato-dextrose agar was introduced, remained free from infection during these tests. The inoculations were made as previously described. In addition, inoculations with culture No. 1 were made on 28 varieties, between 12 and 30 years old (footnote 3, table 5). The number of trees available for inoculation was limited because of the danger of killing valuable trees of bearing age.

TABLE 4.—Cultures of *Phytophthora cactorum* tested for physiologic specialization

Culture No.	Host	Place isolated and year	Collector or source of culture ¹
1	Grimes Golden apple tree.....	Indiana, 1934.....	Writer.
8	do.....	do.....	Do.
12	Apple fruit.....	Baarn, Netherlands.
13	<i>Lilium candidum</i>	Indiana, 1936.....	E. P. Imle.
14	Lilac.....	Massachusetts, 1929.....	K. S. Chester.
15	Peony.....	Indiana.....	Baarn, Netherlands.
16	Citrus.....	Korea.....	Do.
17	<i>Eriobotrya japonica</i>	Japan.....	T. Tasugi.
18	Snapdragon.....	California, 1932.....	M. P. Harris.
19	<i>Pinus</i> sp.....	Baarn, Netherlands.
20	Unknown.....	Do.
21	<i>Lilium dauricum</i>	Japan.....	T. Tasugi.

¹ Cultures Nos. 12 and 14 to 21, inclusive, were received from C. M. Tucker.

Three-week-old flax (Red Wing) plants and peony (Mme. Bou-langer) shoots growing in a greenhouse also were inoculated with the 12 cultures of *Phytophthora cactorum* listed in table 4. A small quantity of mycelium from a culture on potato-dextrose agar was inserted in the stems and covered with a piece of rubber tape. Two groups of 5 plants of flax were grown separately in a greenhouse bench and inoculated with each culture. The tests on peony were conducted in two trials. Five shoots were inoculated in the first trial and 10 in the second. A similar number of check plants treated with sterile potato-dextrose agar instead of inoculum remained free from infection during these tests.

The virulence of the cultures on flax and peony was recorded by assigning values of 0, 1, 2, and 3 to the recognized classes of infection resulting from the inoculations. The value 3 was assigned to the severely infected plants, 0 to the plants not infected, and intermediate values to the plants in the intermediate classes. A virulence of 3 was assigned to the cultures which were pathogenic on the 10-year-old apple trees.

DIFFERENTIAL PATHOGENICITY OF CULTURES

Culture No. 1 isolated from a Grimes Golden trunk was the most widely pathogenic culture on the apple varieties, producing cankers on Grimes Golden, Tompkins King, Northwestern Greening, Rome Beauty, and Stark (table 5). Four other cultures also produced cankers on apple trunks, but differed from culture No. 1 and from one another in the varieties that they infected. Culture 13, from *Lilium candidum* L., was pathogenic on Tompkins King, Rome Beauty, and Stark. Culture 17, from loquat, infected Tompkins King and Smoke-house, being the only culture to infect the latter variety. Culture 14, from lilac, infected only Tompkins King of the 10-year-old trees, but it also infected 19-year-old Grimes Golden in another test. Culture 8, isolated from a Grimes Golden trunk and atypical in growth on media, infected only Gano, and was the only culture to infect this variety. Cultures Nos. 12, 15, 16, 18, and 19 failed to infect any of the above-mentioned seven varieties, which were infected by one or more of the other cultures. Cultures 20 and 21 were nonpathogenic on Grimes Golden. *Phytophthora cactorum* was reisolated from cankers formed by the pathogenic cultures.

Inoculations of flax and peony also brought out differences in pathogenicity of the cultures. Cultures 1, 8, 13, 14, and 17, pathogenic on one or more varieties of apple, were only slightly pathogenic on flax. With the exception of cultures 12 and 15, all of the remaining cultures that were nonpathogenic on apple were highly virulent on flax. All the cultures except No. 15, were highly virulent on peony (fig. 4, F). *Phytophthora cactorum* was reisolated from infected flax and peony plants.

The data presented above are too limited to permit definite classification of races of *Phytophthora cactorum*. However, the writer believes that significant differences in the capacity of cultures of *P. cactorum* to cause collar rot on different apple varieties have been demonstrated.

VARIETAL SUSCEPTIBILITY

TRUNK CANKER

Seven apple varieties were susceptible to phytophthora trunk canker (table 5). The Gano variety was infected by one culture, Grimes Golden by two, Tompkins King by four, Northwestern Greening by one, Rome Beauty by two, Smokehouse by one, and Stark by two. A culture of *Phytophthora cactorum* isolated from peony in 1937, and not included in table 5, was pathogenic on the trunks of 20-year-old Grimes Golden.

No infection resulted on trunks of 10-year-old Arkansas, Baldwin, Ben Davis, Winter Banana, Cortland, Delicious, Oldenburg, Early Harvest, Golden Delicious, Hubbardston, King David, Lowland Raspberry, McIntosh, Maiden Blush, Northern Spy, Paragon, Rhode Island Greening, Stayman Winesap, Starking, Wagener, Wealthy, Winesap, Yellow Transparent, and York Imperial apple trees when inoculated with cultures 1, 8, and 12 to 19. In addition, Benoni, Fameuse, Hibernial, Mann, Red Astrachan, Transcendent crab, Virginia crab, and Walbridge apple trees, 13 to 30 years of age, were resistant to infection by culture No. 1. The inoculations on the trunks from which no infection was recorded, in many cases developed a slight enlargement of the inoculation incision (fig. 3, B). In some cases, only one or two inoculations were made with a given culture on a variety. Therefore, final conclusions on the reaction of a variety to a given culture cannot be made from these tests.

ROOT ROT

Cankers near the bases of the trunks of an own-rooted Grimes Golden, 14 years old, and of an 18-year-old Grimes Golden on French crab seedling roots, extended into the large roots. The writer was unable to determine whether the infected roots arose from the Grimes Golden scion of the latter tree. Apparently pure cultures of *Phytophthora cactorum* were isolated from diseased bark from the roots of both trees. The cankers on the roots had definite margins and the infected periderm was brown and soft. In an orchard at Vincennes, 14-year-old Grimes Golden trees severely girdled by phytophthora trunk cankers were pulled with a tractor (fig. 2, B). The French crab seedling roots of 49 of these trees were healthy. On 1 tree a large root was decayed by an undetermined organism.

FRUIT ROT

Apple fruits of 29 varieties were inoculated with *Phytophthora cactorum* culture No. 1. Two fruits of each variety were taken from cold storage on November 29, 1935, swabbed with 95-percent alcohol, and inoculated by introducing into a puncture a small quantity of mycelium from a young culture. The inoculations were sealed with petroleum jelly. A check puncture was made on the side of each fruit opposite the inoculation. Each variety was enclosed in a waxed paper bag.

Typical decay was produced by all the inoculations and none by the check punctures. After 1 week the infected areas were between 3.3 and 6.3 cm. in diameter on the Baldwin, Ben Davis, Oldenburg, Fameuse, Grimes Golden, Hubbardston, Jonathan, Tompkins King,

Lowland Raspberry, McIntosh, Maiden Blush, Northern Spy, Northwestern Greening, Rome Beauty, Gallia Beauty, Red Delicious, Rhode Island Greening, Stark, Stayman Winesap, Smokehouse, Yellow Transparent, Turley, Wagener, Winesap, Winter Banana, and York Imperial varieties, between 2.2 and 3.3 cm. on Banks and Golden Delicious, and 1.3 cm. on Arkansas.

FACTORS AFFECTING RESISTANCE AND SUSCEPTIBILITY TO COLLAR ROT

RELATION OF AGE AND VIGOR OF GRIMES GOLDEN TREES TO INFECTION

The trunks of Grimes Golden trees ranging in age from 2 to 30 years were inoculated with culture No. 1 (table 2). The 2-year-old trees were nursery-budded and were inoculated the season that they were replanted. These young trees grew vigorously, and were reinoculated twice during each of the following 3 years. No infection occurred until the fourth year. Then one canker resulted from six inoculations on three 5-year-old trees. The marked resistance of the 2- to 4-year-old trees is difficult to explain. Presumably a similar type of resistance occurred when large scaffold branches of older trees were inoculated, as will be shown later. Typical cankers were produced on the trunks of Grimes Golden trees 8 to 30 years old. All the trees were growing well when inoculated, except two 30-year-old trees and a few 14-year-old trees on poor soil. Typical cankers were obtained on the 30-year-old trees, but the small, slow-growing 14-year-old trees on areas of poor soil appeared to be more resistant to infection than more vigorous trees in the same orchard.

Under conditions of natural infection the disease occurs chiefly on Grimes Golden trees over 13 years of age, as mentioned above. However, Grimes Golden trees 8 and 11 to 13 years of age were easily infected when mycelium of the fungus was introduced into the bark of the trunks. It appeared likely that these younger trees escape infection through the operation of some factors other than internal resistance to the parasite. Therefore, a series of inoculations was made by placing inoculum on the uninjured bark of 11- and 19-year-old trees. Colonies of culture No. 1, grown for 2 weeks on prune-extract medium, were placed on the uninjured surfaces of the trunks of Grimes Golden trees, and covered with moist cheesecloth and heavy wrapping paper for 3 days. Check areas were treated similarly, except that no inoculum was used. From nine inoculations made on six, 19-year-old trees, six cankers developed on four trees. One month after inoculation the cankers were 13 to 25 cm. in diameter and showed no evidence of being checked. The fungus was reisolated from four cankers. No infection resulted from four inoculations and two check treatments made on six 11-year-old trees nor from five check treatments on 19-year-old trees. The results show 19-year-old trees to be susceptible to infection from inoculum placed on the unwounded trunk, and they suggest a structural resistance in 11-year-old trees to infection from such inoculation.

RESISTANCE OF BRANCHES

Cankers caused by natural infection with *Phytophthora cactorum* have never been observed by the writer on scaffold branches. To

determine whether the branches are actually resistant or whether they merely escape infection, inoculations were made during 1935 on both the trunks and branches of 22 varieties ranging in age from 12 to 30 years. Mycelium of culture No. 1 of *P. cactorum* was placed in incisions in the bark, as previously described.

Typical cankers resulted on the trunks of 38 of 46 Grimes Golden trees inoculated (table 6). However, only 3 cankers developed from 70 inoculations on branches. One canker, 20 cm. long, encircled a branch 5 cm. in diameter on a 12-year-old Grimes Golden. The canker was sunken, had a definite margin, and the cambium under most of the canker appeared healthy. *Phytophthora cactorum* was reisolated. A second branch canker, 7 cm. in diameter, occurred on a branch 8 cm. in diameter on a 14-year-old Grimes Golden tree. It was definitely checked in growth and delimited from sound bark. A third canker occurred on a main scaffold branch 15 cm. in diameter on a 19-year-old tree. Two months after inoculation the canker was 35 cm. in diameter and still enlarging (fig. 3, C). No infection occurred on the trunks or branches of the other 21 varieties inoculated except Rome Beauty. A trunk canker resulted from inoculations on 2 Rome Beauty trees. From all the inoculations of branches that were classed as no infection, a slight enlargement of the inoculation wound took place (fig. 3, D).

TABLE 6.—Susceptibility of the branches and trunks of apple trees to infection by *Phytophthora cactorum*, culture No. 1

Variety ¹	Age of trees	Trees inoculated	Trunks infected ²	Inoculations on branches		
				Diameter of branches inoculated	Inoculations	Cankers produced
	Years	Number	Number	Centimeters	Number	Number
Grimes Golden-----	12	4	4	5-7	4	1
	13	4	4	4-7	4	0
	14	33	25	5-10	33	1
	19	2	2	7-15	7	1
	19	1	1	2-15	18	0
	20	1	1	11	1	0
Rome Beauty-----	30	1	1	10	3	0
	13	1	1	5-7	2	0
	30	1	0	12	2	0

¹ No infection resulted from a trunk and branch inoculation on 4 Arkansas, 1 Winter Banana, 2 Baldwin, 1 Benoni, 3 Oldenburg, 6 Fameuse, 3 Hibernia, 2 Hubbardston, 4 Jonathan, 2 Tompkins King, 10 McIntosh, 2 Maiden Blush, 1 Mann, 2 Northern Spy, 1 Rhode Island Greening, 2 Red Astrachan, 5 Stayman Winesap, 2 Wager, 9 Wealthy, and 8 Winesap trees, 12 to 30 years of age.

² No infection resulted from 44 check incisions in the trunks.

INTERACTION OF STOCK AND SCION COMPONENTS OF GRAFTED TREES IN RELATION TO RESISTANCE AND SUSCEPTIBILITY

In an effort to avoid trunk cankers, Grimes Golden trees are frequently propagated by grafting the Grimes scion on the stock, 6 or more inches above the soil line, and also by double-working, i. e., by using a second variety to form the base of the trunk onto which the Grimes Golden is grafted. Such trees were used to study the possible effect of stock-scion interaction on the respective susceptibility or resistance of the component parts of the trees to infection by *Phytophthora cactorum*. Fourteen- and twenty-year-old Grimes Golden trees propagated on 13 varieties of stocks, were available for this

test. The Grimes Golden trees, propagated on 5 varieties, had been double-worked in the nursery. The roots of these trees were French crab seedlings. The Grimes Golden scions on 8 varieties of own-rooted stocks had been grafted 8 to 18 inches above the soil line. The roots of the own-rooted stocks were of the same variety as the base of the trunk. Inoculations were made with culture No. 1 near the graft union on the Grimes Golden and also on the stock variety of each tree (fig. 4, D).

Large trunk-cankers were formed on the scion, or Grimes Golden portion, of 56 of the 68 trees inoculated (table 7). Northwestern Greening was the only variety of stock tested that was susceptible, cankers resulting from four of the six inoculations made on four, 20-year-old double-worked trees. No infection occurred from the inoculations on stocks of the varieties Arkansas, Red Astrachan, Delicious, Oldenburg, Fameuse, Hiberna, Lowland Raspberry, Northern Spy, Northern Spy seedling, Transcendent crab, Walbridge, and Wealthy, nor from the check incisions. The downward advance of the cankers on the Grimes Golden bark was checked at the graft union with the resistant stocks. However, when the Grimes Golden was on Northwestern Greening stock, the cankers extended across the graft unions. In this experiment there appeared to be no interaction between the stock and scion which influenced the characteristic resistance or susceptibility of the varieties.

TABLE 7.—*Results of inoculations of stock and scion components of the trunks of high- and double-grafted Grimes Golden trees with culture No. 1 of Phytophthora cactorum*

Varietal composition of the trees ¹		Age of trees	Trees inoculated	Component varieties of trunks inoculated	Inoculations ²	Cankers produced
Intermediate stock	Rootstock					
		Years	Number		Number	Number
Arkansas.....	French crab seedling...	20	8	Grimes Golden.....	8	7
				Arkansas.....	8	0
None.....	Red Astrachan.....	14	1	Grimes Golden.....	1	1
				Red Astrachan.....	1	0
Delicious.....	French crab seedling...	14	6	Grimes Golden.....	6	4
				Delicious.....	5	0
Do.....	do.....	20	10	Grimes Golden.....	10	9
				Delicious.....	9	0
None.....	Oldenburg.....	14	6	Grimes Golden.....	6	3
				Oldenburg.....	7	0
Do.....	Fameuse.....	14	1	Grimes Golden.....	1	1
				Fameuse.....	1	0
Do.....	Hiberna.....	14	3	Grimes Golden.....	3	3
				Hiberna.....	3	0
Do.....	Lowland Raspberry....	14	6	Grimes Golden.....	6	5
				Lowland Raspberry...	6	0
				Grimes Golden.....	4	4
Northwestern Greening.	French crab seedling...	20	4	Northwestern Greening.....	6	4
				Grimes Golden.....	4	2
None.....	Northern Spy.....	14	4	Northern Spy.....	4	0
				Grimes Golden.....	2	2
Do.....	Northern Spy seedling..	14	3	Northern Spy seedling..	3	0
				Grimes Golden.....	4	3
Do.....	Transcendent crab.....	14	4	Transcendent crab.....	4	0
				Grimes Golden.....	5	4
Walbridge.....	French crab seedling...	20	5	Walbridge.....	5	0
				Grimes Golden.....	6	6
None.....	Wealthy.....	14	6	Wealthy.....	6	0
				Grimes Golden.....	2	2
Do.....	Grimes Golden.....	14	1	Grimes Golden.....		

¹ Scion always Grimes Golden

² No cankers resulted from 64 check incisions in trunks of Grimes Golden and 2 in trunks of the Arkansas variety.

CONTROL OF THE DISEASE

EFFECT OF HEIGHT OF THE GRAFT UNION ON INFECTION

During 1933 and 1934 collar rot of Grimes Golden appeared to be more prevalent on low-grafted and budded trees than on double-worked trees. Therefore, measurements were made of the height of the graft unions formed between the Grimes scion and the stock, to determine whether collar-rot cankers were more prevalent on trees with low than with high graft unions. These measurements were made in an orchard of 467 double-worked, 16-year-old trees in which 21 percent were killed by *Phytophthora collar rot*.

The height of the graft unions of the diseased and the healthy trees ranged respectively, from 0 to 22 and from 0 to 24 inches above the soil. The mean height of the graft unions of trunk canker-infected trees was 12.4 inches and that of uninfected trees 12.8 inches. Of 354 trees with graft unions 15 inches or less above the soil, 75, or 21 percent, were infected. Of the remaining 113 trees with higher graft unions, 22, or 19 percent, were infected. Within a group of 15 trees having graft unions 20 to 24 inches above the soil 7 were infected. Evidently the propagation of Grimes Golden trees by grafting 15 to 22 inches above the soil is not effective in reducing natural infection with *Phytophthora cactorum*.

Apple growers interviewed, however, are of the opinion that in general less infection occurs on double-worked trees than on regular grafted and budded trees. It may be that in years when *phytophthora collar-rot* does not occur in epidemic severity, double-worked trees are not so readily infected as low-grafted and budded trees. The use of resistant varieties of apple to form the roots and trunk of the trees, and the grafting of the Grimes Golden variety on these stocks 30 inches or more above the soil, may still be a means of reducing infection from *Phytophthora cactorum*, although no observations have been made of Grimes Golden trees grafted at this height.

BORDEAUX MIXTURE AS A PREVENTATIVE OF COLLAR ROT

The effectiveness of bordeaux mixture as a preventive of collar rot was tested in two orchards. A 16:16:100 bordeaux mixture containing 1 gallon of miscible oil was tested in 1935, and a 30:30:100 bordeaux mixture was tested in 1936 and 1937. The sprays were applied on the trunks of Grimes Golden trees after the dormant period when the apple buds were swelling.

One orchard, at Vincennes, Ind., was 12 years old in 1935. In this orchard the sprays were applied to pairs of tree rows alternating with pairs of unsprayed control rows. There was a total of 151 sprayed and 128 control trees. The other orchard, at Bloomfield, Ind., was 17 years old in 1935. The trees in two-thirds of the rows, comprising 235 trees, were sprayed, while every third row, comprising 135 trees, was left unsprayed as a control. During the years of the test, additional sprays for the control of apple scab and codling moth were applied to all the trees.

Cankers developed from natural infection on 3, 1, and 0.6 percent of the sprayed trees, and on 9, 4, and 0.8 percent of the unsprayed trees in the Vincennes orchard during the years 1935, 1936, and 1937, respectively. In the orchard at Bloomfield, cankers developed on

1, 0, and 0.4 percent of the sprayed trees and on 1, 0, and 1.5 percent of the unsprayed trees, during the years 1935, 1936, and 1937, respectively.

TREATMENT FOR CANKERS BY DECORTICATION

In August 1934, 13 cankers on 14-year-old Grimes Golden trees were treated by decortication. The diseased bark and the healthy bark for 4 cm. beyond the margins of the cankers and the infected and discolored wood beneath the cankers were removed (fig. 4, *E*). The wounds were then painted over with bordeaux-oil paint, as used by Zeller (48). The development of the cankers was permanently checked on 10 trees, while 3 showed slight development in restricted areas.

TREATMENT OF CANKERS WITH ZINC CHLORIDE AND SODIUM ARSENITE SOLUTIONS

The effectiveness of a number of chemical solutions in arresting the development of cankers was tested on Grimes Golden trees from 8 to 25 years old, at Bloomfield, Lafayette, Mitchell, and Vincennes, Ind. The solutions were applied with a brush to the surface of the cankers and to the bark in areas 5 cm. in advance of the cankers. The margins of the cankers when not readily evident were ascertained by probing with a knife, and marked by placing tacks at intervals. In a few treatments with zinc chloride, a thin layer of the outer bark at the advancing margins of cankers was removed with a knife before applying the solution.

Cankers treated with 10-percent copper arsenite in 10-percent ammonium hydroxide, 5- and 10-percent aqueous solutions of sodium arsenite, and Day's (11) solutions containing 43, 53, 64, or 71 percent of zinc chloride in acidified 74-percent alcohol, were not effectively controlled. The cutting away of the outer bark prior to treatment did not improve the effectiveness of the zinc chloride solutions.

Trunk cankers on 93 Grimes Golden trees were treated with a 10-percent solution of sodium arsenite in 50-percent alcohol during the fall of 1935. Only 1 of these cankers showed further enlargement when observed 5 weeks after treatment. Twenty-five untreated cankers continued to enlarge. The cankers were again observed after renewal of growth during the spring. None of the treated cankers showed renewed growth at that time. However, the data on possible renewed growth in the spring is inconclusive, since only 2 of the 25 untreated cankers renewed growth. The failure of the untreated cankers to enlarge in 1936 was an unusual occurrence, possibly associated with the effects of extremely low temperatures during January 1936. Of 13 cankers treated with the alcoholic sodium arsenite solution in 1936, 7 showed continued development 5 weeks after treatment. During 1937, 6 cankers were treated with this solution. Only 1 of these showed renewed activity in a restricted area when examined 6 weeks after treatment and again in 1938. Two untreated cankers continued to enlarge in 1937, and renewed enlargement in 1938. No deleterious effects of the solution on the upper portions of the trees were noticed during a period of 2 years after treatment. The solution was injurious to the bark, but the injury in all cases was confined to the treated area. The callus at the margins of treated cankers appeared to be retarded on a few trees.

DISCUSSION

Collar rot of uncertain origin on Grimes Golden and other varieties of apple has been reported by a number of investigators (15, 23, 37, 43) during the years from 1900 to 1921. The symptoms described in many cases are similar to those of *phytophthora* collar rot. Furthermore, the varieties Grimes Golden and Thompkins King, often described by earlier workers as especially subject to collar rot, are shown to be most susceptible to *phytophthora* collar rot in the present work. The writer therefore believes that very likely many of these early reports deal with the *phytophthora* collar rot disease and that it has been an important cause of loss for many years.

The fact that the cankers in the incipient and actively enlarging stages are not easily detected by casual observation, may account for the failure of earlier workers to detect the causal relation of *Phytophthora cactorum* in the collar rot disease. Infected trees usually do not exhibit prominent symptoms until late in the fall and the second year after infection, at which time the cankers may not be very active. The causal fungus has not been obtained in culture from killed bark which had become dried and pulled away from the wood, and was isolated with difficulty from old cankers which were slightly active or had recently ceased enlarging.

In the physiologic specialization studies a limited number of inoculations were made with each of the 10 cultures tested on 30 varieties of apple trees other than Grimes Golden. Very likely more of the varieties would have been shown to be susceptible to one or more of the cultures had a larger number of inoculations been made. However the writer believes that the apparent differences in pathogenicity and selectivity of host variety exhibited by the cultures cannot be explained entirely on the basis of escape from infection. There is the possibility that the virulence of the cultures is altered with time on artificial media. This may have occurred in the case of culture No. 15, which was isolated from peony. The differences in pathogenicity of all the cultures on the varieties of apple trees, however, cannot be interpreted as traceable to a degeneration of pathogenicity of the cultures, since a number of cultures differing distinctly in pathogenicity were of recent isolation. Thus, cultures Nos. 1 and 8 were isolated in 1934, No. 13 in 1936, No. 14 in 1929, and No. 18 in 1932. Furthermore, cultures Nos. 12, 16, and 18 to 21, inclusive, which were non-pathogenic on 31 varieties of apple trees, were pathogenic on flax and peony plants. Müller (28) has shown that there are also physiologic races of *Phytophthora infestans* (Mont.) De Bary.

Grimes Golden trees, 2 to 4 years old, are resistant to infection even when inoculum is inserted in the bark. However, this resistance in the trunk is lost as the tree matures. Trees of resistant varieties are resistant at all ages. Bearing Grimes Golden trees with rough bark on their trunks are susceptible to infection by inoculum placed both on the surface of the trunk and in incisions in the bark. However, the large branches of bearing Grimes Golden trees are highly resistant to infection when the inoculum is inserted in the bark. The resistance of the large branches may be the same as that possessed by the young trees.

The component varieties of double- and high-graft Grimes Golden trees maintained their own specific reaction toward infection by

Phytophthora cactorum. This is in accord with the results obtained by Bond (6), Leach (24), May (27), Roach (33), and Salmon and Ware (35) in their respective investigations of the interaction of the stock and scion component parts of grafted plants on susceptibility and resistance to specific diseases. On the other hand, Hofmann (21), Richmond (32), and Wormald and Grubb (47) obtained evidence of an altered susceptibility or resistance of the stock and scion parts of grafted plants or their progenies to infection by specific organisms.

The most promising means of controlling the phytophthora collar rot disease of apple trees is the propagation of the susceptible varieties by grafting on desirable varieties of stocks that are resistant to the disease. The graft union of the susceptible variety with the resistant stock probably should be at least 30 inches or more above the soil line. From the standpoint of protection from phytophthora canker and from cold injury to the trunks and crotches of the trees, the more desirable practice would be to graft the susceptible varieties on the main branches of young trees of resistant varieties, after they have become established in the orchard.

Bordeaux mixture applied to the trunks of apple trees was only partly effective in preventing collar-rot infection. Fawcett (13, 14) has recommended scarifying cankers and painting the resulting wounds with bordeaux paste for the control of phytophthora gummosis of citrus trees. He also advocated painting the trunks with bordeaux paste, and the application of a dust of zinc sulphate, copper sulphate, and hydrate lime (12 : 1 : 6) around the bases of young citrus trees, to prevent infection. Curzi (10) recommended decortication of infected tissues and applications of 3 to 5 percent bordeaux mixture as a treatment of phytophthora crown-rot cankers of peach.

SUMMARY

Phytophthora trunk canker, or collar rot, in 1933-34 caused serious losses in apple orchards in Indiana. In six orchards, from 21 to 68 percent of the Grimes Golden trees 14 to 18 years old were infected. In two of these orchards examined each year from 1935 to 1937, annual infection ranged from 0 to 6 percent. Evidence is presented in the review of literature which indicates that loss of Grimes Golden trees from phytophthora collar rot occurred in Indiana as early as 1900.

The disease is not easily detected in its early stages. The first outward symptom is a moist, discolored area on the surface of the bark. The infected bark is dark colored, has a strong fermented odor, and in later stages becomes dry, cracked, and drawn away from the wood. Frequently trees are girdled during one season.

Phytophthora cactorum is shown to be the casual agent of the Grimes Golden collar rot disease. The mycelium of the causal fungus develops both intercellularly and intracellularly, and penetrates all the tissues of the bark. Cultures of *P. cactorum*, pathogenic on apple trees, were isolated from active cankers, diseased fruits, and orchard soil. The fungus was also isolated from sapwood beneath a canker.

Evidence was secured of the existence of physiologic races of *Phytophthora cactorum*, which differ in their ability to infect varieties of apple trees. Certain cultures also differed in degree of virulence on the Grimes Golden variety. The Gano, Grimes Golden, Tompkins

King, Northwestern Greening, Rome Beauty, Smokehouse, and Stark varieties of apple trees were susceptible to one or more physiologic race of *P. cactorum* when artificially inoculated. Certain cultures of *P. cactorum* also were pathogenic on cherry, peach, and plum trees, and on flax and peony shoots. All 29 varieties of apple fruits tested were susceptible to fruit rot when artificially inoculated.

Grimes trees, 2 to 4 years old, were highly resistant both to artificial and to natural inoculation with *Phytophthora cactorum*. Older trees, 8 to 30 years old, were highly susceptible to infection from artificial inoculation. In the field the disease seldom occurs on trees less than 13 years of age. Differences also were found in the ease with which parts of the same tree were infected when inoculated. Trunks of bearing Grimes Golden trees were readily infected, while large branches were only occasionally infected when artificially inoculated.

The component varietal portions of double- and high-grafted Grimes Golden trees retained their specific resistance or susceptibility to *Phytophthora cactorum* unaltered by stock or scion influence.

The practice of grafting Grimes Golden scions on stocks at heights of 15 to 22 inches above the soil was found to be inadequate for the prevention of collar rot infection.

Bordeaux mixtures of the 16:16:100 and 30:30:100 formulae, were found to give partial control of collar rot infection. Day's solutions containing 43, 53, 64, or 71 percent zinc chloride were found to be ineffective in eradicating cankers on Grimes Golden apple trees. A 10-percent solution of sodium arsenite in 50-percent alcohol gave promise of being an effective means of checking the development of established cankers.

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GROWTH AND DISTRIBUTION OF ROOTS OF THE PERFECTION PIMIENTO IN GEORGIA¹

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INTRODUCTION

The pimiento, or mild-flavored type of pepper (*Capsicum frutescens* L. var. *grossum* Bailey), is the most important vegetable cash crop grown in the lower piedmont section of Georgia, and from the standpoint of both value and quantity of pack, it ranks high among the commercial canning crops of the State. The acreage planted to pimientos in Georgia increased more than 35 percent from 1937 to 1938.

Growers often find difficulty in getting good stands of plants that will live and produce fruits until frost. Many of the plants are killed by the fungus *Sclerotium rolfsii*, but not all the losses can be attributed to this organism. Observations during the last four growing seasons have led the writer to conclude that another important contributing factor is the burning of the roots by the fertilizer applied. The method of fertilizer application may, therefore, be of considerable practical importance to the grower, but before this problem could be considered it was necessary to make a study of the development and distribution of the roots in the soil. The results of the study are reported in this paper.

MATERIALS AND METHODS

The study was conducted in the horticultural plots of the Georgia Experiment Station, which is situated in Spalding County, the center of the pimiento industry in Georgia. Although mechanical analysis shows the soil on which the plants were grown to be a sandy loam, it closely approaches the texture of a sandy clay loam, Cecil series, as interpreted by the classification of Davis and Bennett.³ Both of these are predominant soil types of the pimiento-growing area.

A selected strain of Perfection pimiento seed was planted in an electrically heated hotbed on February 10, 1938. By February 20 most of the seeds had germinated and small plants were showing above the soil. In order to obtain a progressive knowledge of the development and distribution of the roots, an examination was made at the end of each 30-day period beginning February 28, 7 days after the seeds were up, and continuing through October 28, when the plants were approximately 8 months old. The first three examinations were made while the plants were growing in the hotbed. This was done by carefully removing the loose composted soil from around the roots with a hand fork and with water under light pressure from a garden hose.

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² The author is indebted to M. M. Murphy, Jr., of the Department of Horticulture, for taking the photographs used in this paper, and to L. C. Olson, of the Department of Agronomy, for assisting in taking the soil samples and for making the mechanical analyses.

³ DAVIS, R. O. E., and BENNETT, H. H. GROUPING SOILS ON THE BASIS OF MECHANICAL ANALYSIS. U. S. Dept. Agr. Dept. Cir. 419, 15 pp., illus. 1927.

A few days prior to setting the plants in the field the soil was thoroughly prepared and fertilized with 600 pounds per acre of a 4-8-6 (N-P-K) fertilizer placed under the plants, as is the usual method of application for this crop. In order to facilitate handling and tracing the root system, two methods of spacing the rows and plants were used. The plants to be examined during the first 2 months after transplantation were set 5 feet apart in the row and the rows were spaced 5 feet apart. The rest of the plants were set 10 feet apart in rows spaced 10 feet apart, thus allowing ample room for root development in all directions. To avoid disturbing the roots during the growing season, weeds and grass were kept down by frequently scraping the plots with hoes rather than by cultivating them with plows.

The direct or digging method of examination, with the use of water under pressure in some cases to uncover the smaller roots, was employed in this work. (See fig. 8.) The method consisted of digging a trench by the side of the experimental plants to a depth below the deepest roots and of sufficient size to permit two persons to move about in it. The trench afforded a rather smooth perpendicular wall surface into which one could dig with a sharp hand pick and uncover and trace the root system. Several plants were examined in this manner each month. In some cases, especially on the older and larger plants, there were so many rootlets that it was impossible to show all of them in the drawings of the root systems. When this occurred a circular excavation (see fig. 13) was made around the plant, the inner face of which was 8 inches from the main stem of the plant, and the inner surface of the excavation was marked off into square feet with string. The root ends were then charted on coordinate paper in as nearly their exact position as it was possible to locate them.

Air temperature records were taken with a calibrated hygromograph at a point 12 inches above the soil among the plants, while the soil temperature was taken with a calibrated soil thermograph the bulb of which was placed among the roots 4 inches below the soil surface. Rainfall and evaporation records were secured from the nearby experiment station weather station.

Soil samples for mechanical and quick chemical analysis were taken from 20 holes dug at random over the field where the studies were being made. The holes, which were 36 inches wide, 54 inches long, and 36 inches deep, were large enough to permit accurate samples to be secured and further studies to be made of the various profile horizons in relation to root growth and distribution.

ROOT GROWTH IN THE HOTBED

Immediately after germination of the pimienta seed the young primary root typically grows directly downward. It penetrates the warm friable soil of the hotbed readily and by the time the plant is a week old it has usually reached a depth of 3 to 6 inches (fig 1, A). At this stage in the growth of the primary root no lateral or secondary roots can be detected. However, a few subsequent daily examinations show that they make their appearance within 8 to 10 days after germination, depending to a large extent on the moisture content and temperature of the soil. The first secondary roots originate at the upper extremities of the primary or taproot, and others develop

in rapid succession throughout its entire length with the exception of the immediate end.

At the time of the second monthly examination of the root system in the hotbed (March 28), it was found that most of the secondary roots were confined to about the first 3 or 4 inches below the soil surface (fig. 1, *B*). These roots extended outward in a somewhat horizontal direction to a distance of 6 inches on either side of the plant for a total spread of 1 foot, while the primary or taproot had increased in length to about 10 inches. Other lateral roots below this particular group had produced tertiary roots, all of which tended to penetrate the soil in a rather oblique or vertical direction.

By April 28, the last date on which the roots were examined in the hotbed, the lateral or secondary roots had increased in number, length, and size, as compared with those of the previous month (fig. 1, *C*). Some had reached a length of slightly over 10 inches, were very much branched, and averaged 0.6 mm. in diameter. The

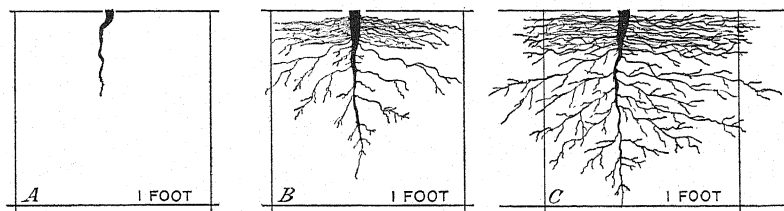


FIGURE 1.—Young primary root (*A*) of pimiento 7 days after germination and root system of the young pimiento plant 37 (*B*) and 68 (*C*) days after germination.

growth of the primary or taproot during this 30-day period, however, was only about one-half as much as that made by the laterals.

GROWTH AND DISTRIBUTION OF ROOTS IN THE FIELD

The heating units were turned off in the hotbed on March 31 and the plants allowed to harden. The total amount of rainfall in April exceeded by far the total amount of evaporation for the same period (table 1); thus the soil in the field remained in good physical condition and was well supplied with moisture during the entire month.

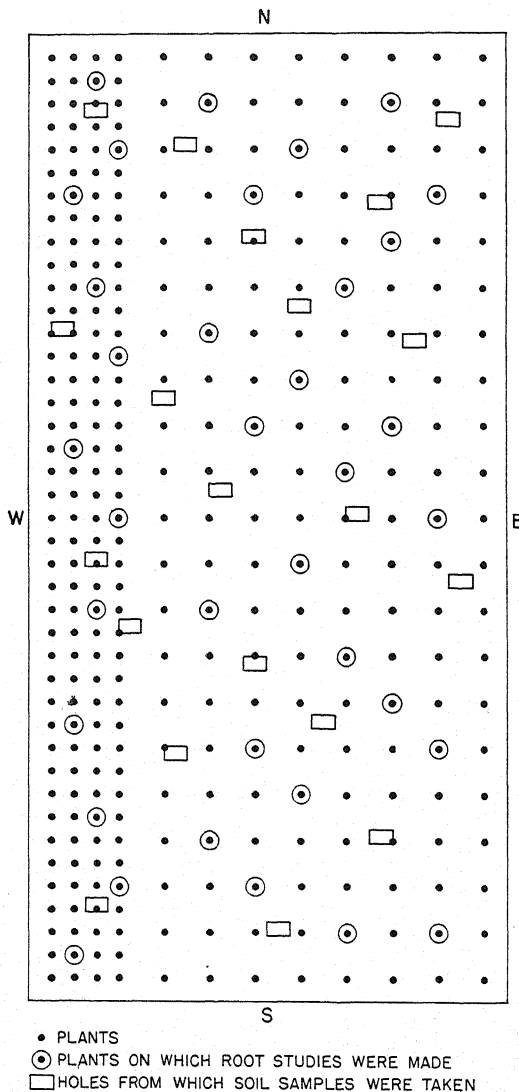
TABLE 1.—Total monthly rainfall, evaporation, and rainfall-evaporation ratio during the 1938 growing season at Experiment, Ga.

Month	Total rainfall	Total evaporation	R/E ratio	Month	Total rainfall	Total evaporation	R/E ratio
	<i>Inches</i>	<i>Inches</i>			<i>Inches</i>	<i>Inches</i>	
April.....	10.46	4.79	2.183	August.....	2.00	6.14	.325
May.....	3.14	7.05	.445	September.....	1.16	5.62	.206
June.....	8.48	7.39	1.147	October.....	.26	5.71	.045
July.....	5.88	6.34	.927				

The plants were carefully taken up from the bed and transplanted to the nearby field, as shown in the lay-out in figure 2, during the late afternoon of April 28. At this time they were about 68 days old, averaged 8 inches in height, and had many small flower buds. Wea-

ther conditions were ideal for the transplanting, and the plants scarcely wilted after being set out, but later it could be seen that the move had distinctly checked their growth.

The first examination of the root system in the field was made on May 27. Here, too, the effects of transplanting were evident in



roots. These findings concerning the effects of transplanting young pepper plants on lateral root formation are in complete agreement with those of Weaver and Bruner,⁴ who studied the development of the root system of the Bell, or Bull Nose, variety of pepper under

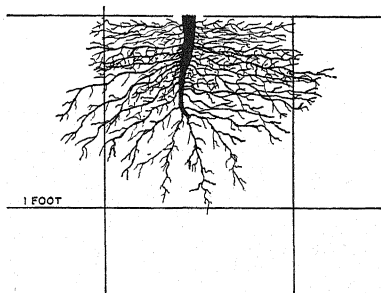


FIGURE 3.—Root system of pimiento 30 days after being transplanted to the field.

field conditions at Norman, Okla. On May 27, the vertical penetrating roots were found to extend 14 inches deep. These roots, together with their young laterals and the ones from the old taproot immediately above, almost completely filled the surface foot of soil at the end of the first 30-day period in the field.

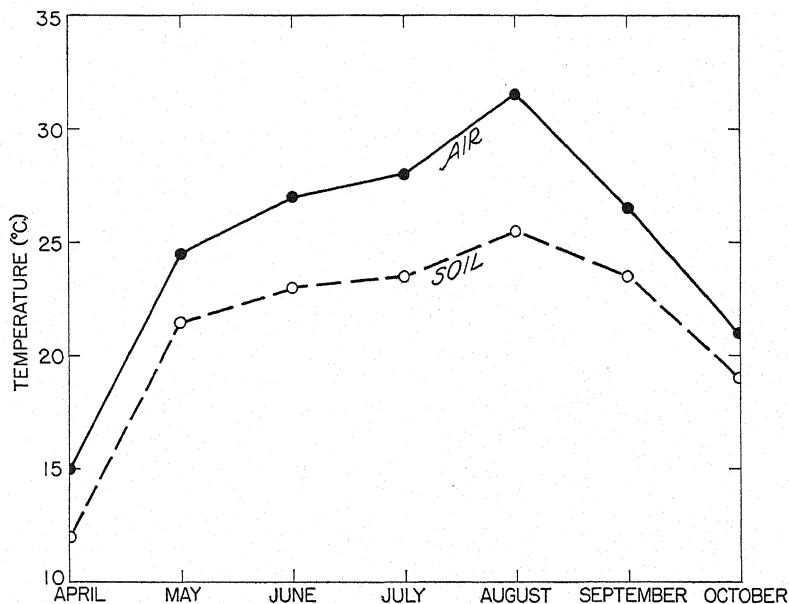


FIGURE 4.—Air and soil temperatures in the pimiento field during the experiment.

With approximately $8\frac{1}{2}$ inches of rainfall during June and an average atmospheric and soil temperature of 27° and 23° C. respectively (table 1 and fig. 4) conditions for plant growth were relatively good.

⁴WEAVER, JOHN E., and BRUNER, WILLIAM E. ROOT DEVELOPMENT OF VEGETABLE CROPS. 351 pp., illus. New York. See pp. 268-273.

A second field examination was made on June 27. The plants at this stage averaged 16 inches in height and had a spread of 22 inches. There were many buds and blooms and also a few green fruits, the largest of which scarcely exceeded 7 mm. in diameter.

The stem was 13 mm. in diameter at the surface of the soil and tapered gradually to a rather blunt root end which extended 6 inches in depth, the taproot having been broken in transplanting. As will be recalled, in the above examinations the prominent part of the root

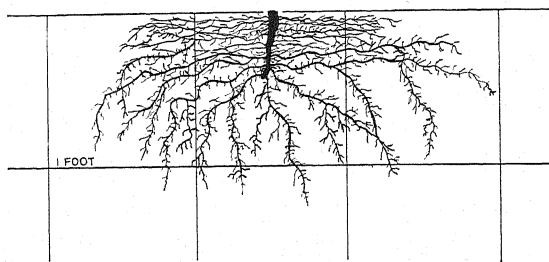


FIGURE 5.—Root system of the pimienta 60 days after being transplanted to the field.

system consisted of many laterals. Some of these had now extended outward 18 inches, mostly in the A horizon or surface 6 inches of soil, and measured 1.5 mm. in diameter. Other shorter ones ran for distances of 4 to 12 inches. They had branched at the rate of 4 to 10 laterals per inch and ranged from $\frac{1}{2}$ to $1\frac{1}{2}$ inches in length. Nearly all the larger roots extended horizontally or slightly obliquely downward for some 10 to 14 inches and then turned downward and ran vertically or again obliquely to a maximum depth of 16 inches (fig. 5).

By the date of the third field examination, July 28, the plants had

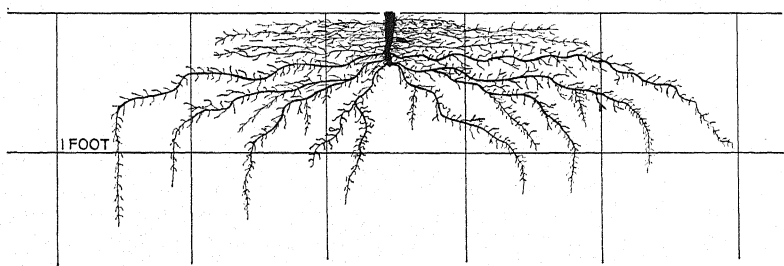


FIGURE 6.—Root system of the pimienta 90 days after being transplanted to the field.

attained an average height of 26 inches and a spread of 25 inches. The old stubby portion of the taproot measured only a little more than 5 inches in length but had a large number of lateral roots growing out from it in all directions, some as long as 30 inches. The majority of these roots, however, were confined to a radius of 24 inches. The larger ones were attached near the end of the old primary or taproot (fig. 6) and measured 3 mm. in diameter, while the smaller secondary roots, although relatively short and averaging but 1 mm. in thickness, were numerous and very well branched. Some of them were found

within the first inch of topsoil. As during the previous examination it was found that the longer roots, after running in a horizontal direction for 18 to 20 inches, turned downward in an oblique and some in a distinctly vertical direction. The vertical penetrating roots had at this stage reached a maximum depth of 20 inches. One small root, however, was traced to a depth of 26 inches but was not included in the drawing because a closer examination revealed that it had not penetrated the subsoil normally but had followed the course of a channel made by the root of a pecan tree that in former years had grown close by.

As shown in table 1, only 2 inches of rain fell during August, while the total evaporation for the month was 6.14 inches, giving a rainfall-evaporation ratio of 0.325. The accompanying average air temperature was 31.5° C., the highest recorded for the entire growing season. During this 30-day period the plants grew less than 2 inches in height, although it will be seen below that root extension proceeded at a rapid rate.

The fourth field examination of roots was made on August 27. The taproot at this stage of growth was 45 mm. thick at the soil

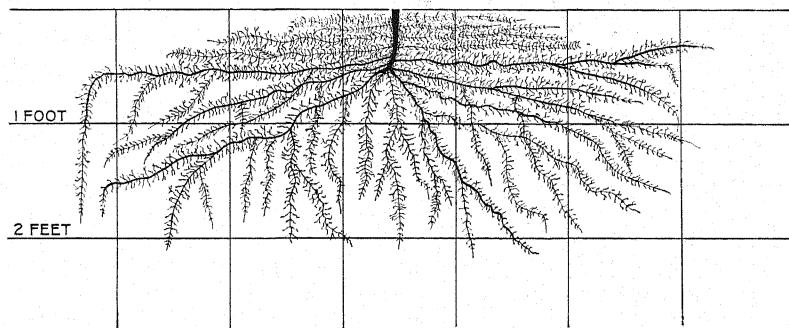


FIGURE 7.—Root system of the pimiento 120 days after being transplanted to the field.

surface, tapered to a diameter of 13 mm. at the end, and gave rise to many laterals. The previously small roots found in the surface 6 inches of soil had increased greatly in both diameter and length. Many were as much as 1.5 mm. in thickness and extended horizontally for a distance of 24 inches. The larger roots near the base of the taproot had made even greater growth (fig. 7). From 6 to 10 were found that measured as much as 6 mm. in diameter. Even as far as 2 feet from the base of the plant some of these strong roots were 2.5 to 3 mm. thick. The maximum lateral spread of the root system had increased from 29 inches in July to 40 inches in August for a total gain in root length of 11 inches. It will be noted further in figure 7 that nearly all the larger and more deeply penetrating roots again ran horizontally for about 24 inches and then turned obliquely downward near their extremities or else abruptly downward and paralleled the course of the old taproot. However, the vertical growth at this stage, reaching a little more than 2 feet in depth, had not kept pace with that horizontally, due in all probability to the difference in soil texture and compactness. Some properties of the A and B horizons of the Cecil sandy loam soil are shown in table 2.

From the large vertically growing roots mentioned above numerous smaller roots originated, and most of these grew in the same vertical direction. These roots ranged in length from 6 to 18 inches and



FIGURE 8.—Lateral root growth in the A soil horizon of the pimienta 150 days after being transplanted to the field.

averaged approximately 0.6 mm. in diameter. Some had rebranched and all were covered with young rootlets at the rate of three to eight per inch. This network of roots was, no doubt, very effective in absorbing moisture.

TABLE 2.—*Some properties of a typical Cecil sandy loam soil profile in the pimiento-growing district of central Georgia, 1938*

Profile layer	Depth of layer	Description of layer	Com- bined sand	Clay	Silt	pH
A.....	Inches 0-6	Brownish-red sandy loam.....	Percent 69.9	Percent 17.2	Percent 12.9	4.89
B.....	6-50	Red, stiff clay.....	45.6	45.7	8.7	4.76

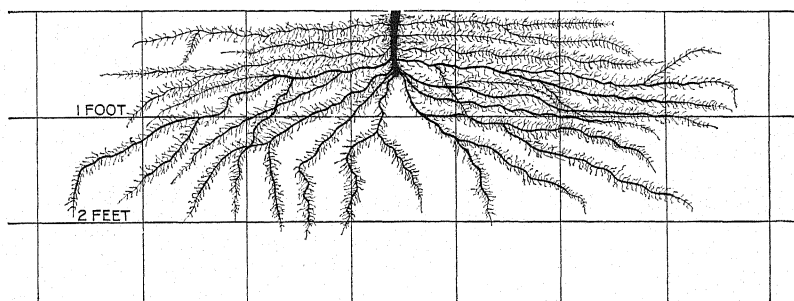


FIGURE 9.—Root system of the pimiento 150 days after being transplanted to the field.

By the time the September examination was made the plants had been growing in the field for 5 months. Owing to a prolongation of the

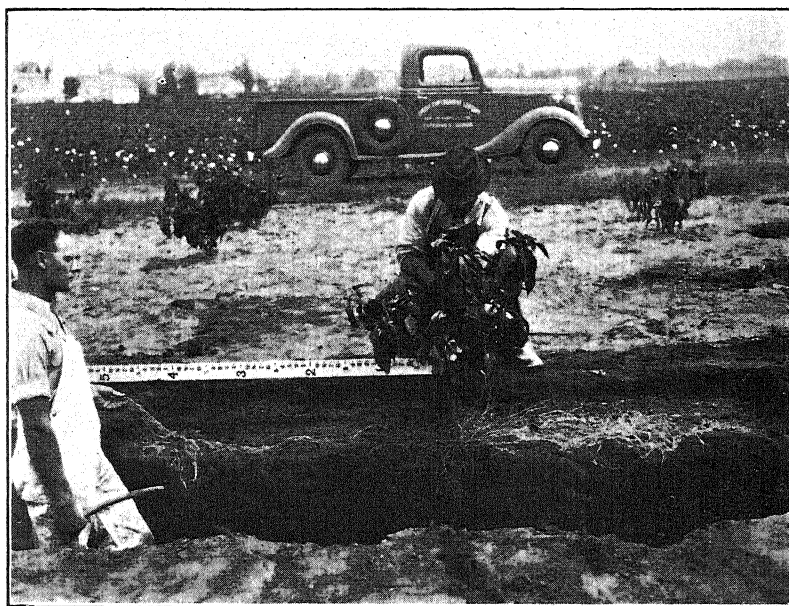


FIGURE 10.—Extent of lateral root growth of a mature pimiento plant.

drought and hot weather, the top growth was very little greater than that of the previous month, but root growth was still making progress. The smaller and more fibrous lateral roots of the A horizon

now extended outward as far as 32 inches from the base of the plant and were so numerous that they completely filled that portion of the soil (fig. 8). Many of them were relatively close to the soil surface. The larger, horizontally growing roots from near the base of the

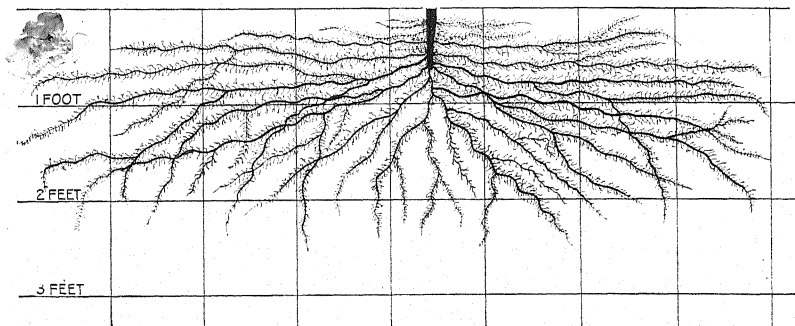


FIGURE 11.—Part of the root system of the pimienta 180 days after being transplanted to the field.

old taproot made about 6 inches if growth in September. They now extended outward 36 inches on all sides of the plant, having a total spread of 6 feet, and some measured as much as 10 mm. in diameter at the largest point. As can be seen in figure 9, however,

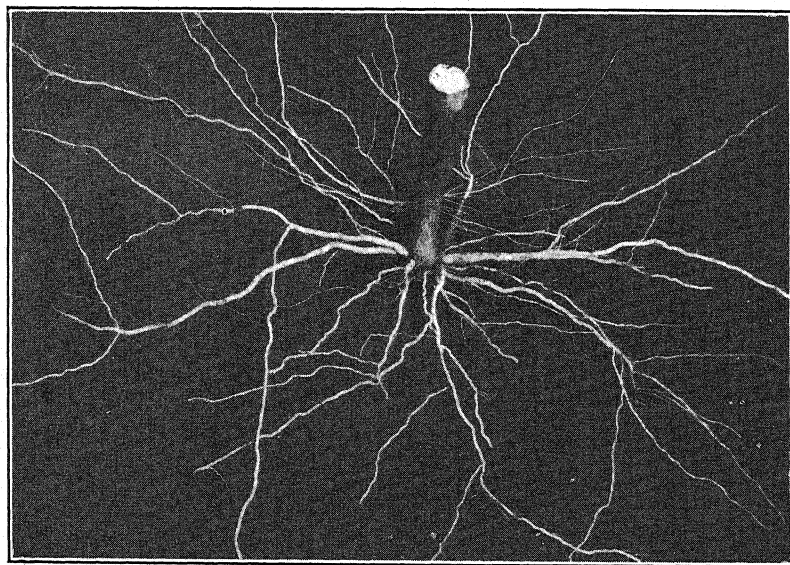


FIGURE 12.—Upper part of the primary root of a mature pimienta plant showing absence of laterals.

the progress made by the roots in penetrating the heavy clay-subsoil was small as compared with that of similar roots examined a month earlier.

As the average date of the first killing frost in central Georgia is November 10, the final monthly examination of roots was made on

October 28. The plants averaged 34 inches in height and 29 inches across. They were loaded with fruits of all ages and some were still blooming. As is shown to some extent in figure 10, and more in

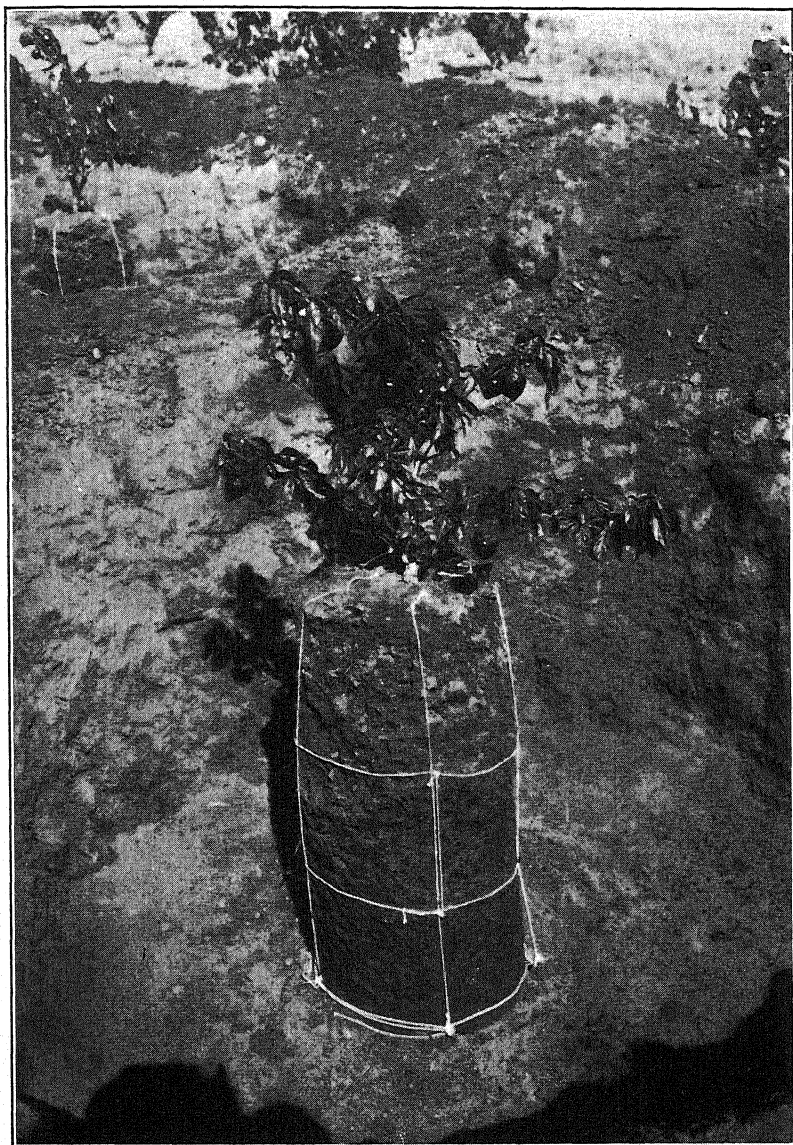


FIGURE 13.—Mature pimiento plant removed by the circular method of excavation.

detail in figure 11, the root system now extended outward to a distance of 48 to 52 inches from the base of the plant. As a rule the horizontally growing roots were a few inches longer when growing parallel with or in the immediate row channel than when at right angles

to it. The larger and more prominent of these roots usually turned downward about 30 inches from the place of attachment and extended well into the second foot of soil. The distinctly vertically penetrating roots generally extended a few inches deeper. Thirty-two inches was the maximum depth at which any roots were found during the experiment and only a few of these appeared in the October excavation.

It is to be noted particularly in figures 11 and 12 that lateral roots are not always initiated throughout the entire length of the primary or upper portion of the taproot, especially in plants that are grown on heavy clay-loam soils. Neither deep setting nor high mounding of the plants under field conditions was found to have any noticeable

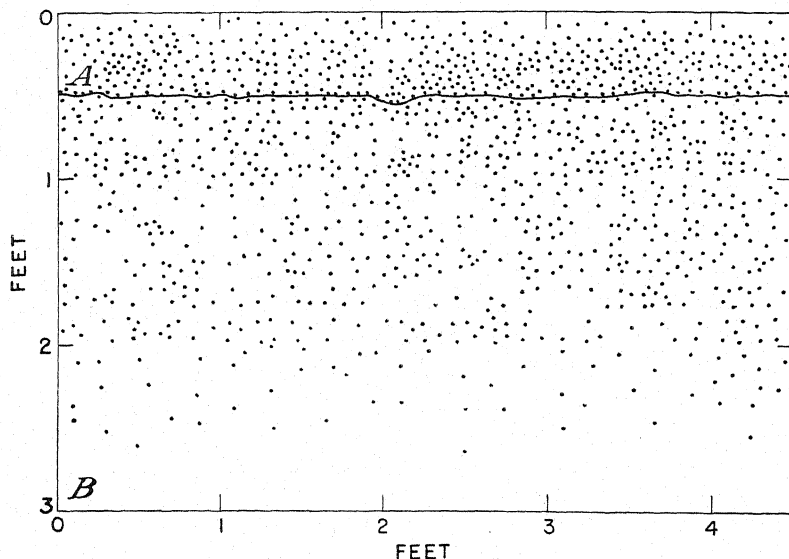


FIGURE 14.—Root concentration and penetration of a mature pimienta plant in two soil profile horizons. Each dot represents a root end.

effect on lateral root initiation at points above those at which they ordinarily occur.

The second method employed for studying the distribution of roots of mature plants (figs. 13 and 14), which is similar to that used on deciduous fruit trees by Oskamp,⁵ also showed that the pimienta roots thoroughly occupy the soil from within a few inches of the surface to a depth of 24 inches. Some roots extend deeper, as was found by the first method of study, but with an increase in depth below 24 inches there is a corresponding decrease in both number and size of roots.

In general conformation the root system of the Perfection pimienta as found in this study is much the same as that reported by Weaver and Bruner⁶ for the Bell, or Bull Nose, variety of pepper. The results of this study also substantiate in large measure those obtained by Mohammad and Deshpande⁷ in similar work on chilies in India.

⁵ OSKAMP, JOSEPH. THE ROOTING HABIT OF DECIDUOUS FRUITS ON DIFFERENT SOILS. Amer. Soc. Hort. Sci. Proc. (1932) 29: 23-219, illus. 1933.

⁶ See footnote 4.

⁷ MOHAMMAD, ALI, and DESHPANDE, R. B. STUDIES IN INDIAN CHILIES. 2 THE ROOT SYSTEM. Agr. Jour. India 24: 251-258, illus. 1929.

DISCUSSION

At present the usual spacing of pimiento plants in central Georgia is 18 to 36 inches in rows 36 to 42 inches apart. Within the first 3 months after the plants are transplanted to the field their roots thoroughly occupy the soil both in and between the rows and many of them are relatively close to the surface. Largely as a matter of custom pimiento growers cultivate their plants regularly about every 2 weeks whether there are weeds to be killed or not. There is little justification for this practice, for it cuts nearly all of the shallow feed roots between the rows and makes it impossible for the plants to utilize the maximum amount of nutrients in the soil. The effects of this practice are especially detrimental in late dry seasons when the plants are loaded with mature fruits. Weed-control measures should be instituted if possible before the weeds can become established and compete with the crop for moisture and nutrients. When no weeds are present and a soil mulch has formed, cultivation is an unnecessary expense.

As much as 100 to 200 pounds per acre of extra commercial fertilizer is usually applied as a side dressing around pimiento plants twice during the growing season, the first about June 1 and the second in early September. In the light of the results of this investigation, it appears that a greater utilization of the fertilizer may be accomplished if it is scattered over the entire area between the rows, rather than in bands near the base of the plants.

SUMMARY

As soon as the pimiento seed germinates in the hotbed, which usually takes about 10 days, the young primary root grows typically directly downward. After 2 months in the hotbed, with relatively good care, the plants are well hardened and ready to be transplanted to the field. At this stage the primary root extends down to about the 10- to 12-inch level and is well supplied with laterals measuring from 4 to 10 inches in length and about 0.6 mm. in diameter.

The primary or taproot is usually more or less damaged in the process of transplanting. The remaining short portion of the taproot does not appear to make up a very important part of the root system as such, but from this and the base of the stem arise many laterals, except in some cases on heavy clay soils, that continue to develop and eventually constitute a very efficient absorbing system. These roots grow horizontally outward to vertically downward and by the time they have been in the field for 60 days they completely occupy the soil on all sides of the plant to a depth of 10 to 14 inches. By the last of August the larger and more deeply penetrating roots extend outward 40 inches from the base of the plant and downward as deep as 26 inches. Mature or 8-month-old plants have a root spread of 48 to 52 inches on each side, many of the laterals being found in the second foot of the soil. Relatively few roots penetrate the stiff clay subsoil deeper than 24 inches.

A STATISTICAL STUDY OF WINTER PAUSE IN WHITE LEGHORN PULLETS ¹

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INTRODUCTION

The character commonly referred to as winter pause has been recognized for a long time. However, its nature or the factors bringing about its expression are still largely unknown. Goodale (1, 2) ³ considered that both hereditary and environmental factors were responsible. In a later report, Goodale and Sanborn (3) found that duration of pause could apparently be reduced by selection. On the other hand, indication of seasonal incidence was seen in the fact that 90 percent of the birds in their flock that started to lay in September exhibited winter pause, while of those that started to lay in December only 30 percent paused. Hays seems to be the only other worker who has pursued extensive studies on the subject. As early as 1924 he (4) suggested a single recessive factor for 7-day or longer pauses. In 1926 Hays and Sanborn (7) presented correlations between the length of winter pauses of 4 days or more and hatching date (-0.2480), date of first egg (-0.3205), age at first egg (-0.2329), and length of the laying period before the pause (-0.1385 , the correlation ratio being 0.2199).

In 1936 Hays (6) presented similar correlations as well as correlations between winter-pause duration and a whole series of other production factors. Most of these, however, illustrate relations with post-pause characters. North (8) found that pausing caused an increase in body weight and two or more pauses an increase in egg weight.

The present study attempts to analyze statistically the nature of winter pause and its expression.

MATERIAL AND METHODS

In a study of such an ill-defined character as winter pause, a series of arbitrary definitions must be set up. Although any conclusions reached will of necessity apply only to the populations considered and to the factors defined at the outset, a better understanding may ensue from studies of this type. The writers believe that, while studies on experimental physiology will ultimately explain the mechanism of operation of the factors determining annual production, further preliminary biometric studies to point the way to experimental verification are needed.

Accordingly, two populations of Single-Comb White Leghorn pullets were selected from the station flock. The J population included 768 pullets hatched in 5 groups at intervals of 1 week, beginning March 20, 1934. The K population consisted of 626 pullets hatched in 4 groups

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² Assistance in calculation of statistical constants was provided by the Works Progress Administration, official project A. P. No. 465-03-3-209.

³ Italic numbers in parentheses refer to Literature Cited, p. 210.

at weekly intervals, beginning March 19, 1935. The basis of selection of both groups from pullet flocks numbering about 1,200 in the J series and 1,100 in the K series consisted of the following criteria, only birds answering these requirements being included:

- Birds maturing before January 1.
- Birds alive on July 1 of their second year of life.
- Birds which did not exhibit broodiness before March 1.
- Birds which were not floor layers at any time.
- Birds which, if they paused before March 1, resumed laying before July 1.
- Birds which had at least 19 sisters or half-sisters by a common sire, answering all of the above requirements.

Twenty sire families constituted the J series and 17 sire families the K population.

Winter pause is defined as a continuous nonlaying period of 7 days or longer beginning after at least one egg was laid, and before March 1. The factors studied were: (1) Percentage of the birds in a sire family, in a hatch, or in a series, which exhibited one or more winter pauses; (2) average duration of the pauses; (3) frequency of occurrence of pauses per pausing bird; (4) degree of pausing, which represents the loss in productive days due to pause in any given period; and (5) date and age on the first day of pause.

The degree of pausing, calculated as the percentage of pausing hens in any given period, is considered as an index expressing the extent of pausing in any of the groups considered. The degree of pausing was calculated for twelve 10-day periods from November 1 to February 28, inclusive. It is important to note that this includes all pauses as here defined irrespective of the causative factors involved, of which many may be in operation.

Prepause factors considered in this study include the date of hatch, the date of first egg, and the age at first egg of all the pullets in a sire family and of the pullets which later exhibited winter pause.

The total degree of pausing in the two populations differed markedly, the K series showing only somewhat over half the pausing observed in the J series. The data for the latter will be presented first, and only such K series data as do not show agreement with the observations on the J population will be given.

DATA ON THE J SERIES

Data on the J series were analyzed from two standpoints: (1) As one population, irrespective of descent, and (2) as 20 separate sire families in order to determine the genetic implications of the relation between the different factors studied.

Table 1 presents the pausing characteristics of this population with respect to date of hatch. The degree of pausing is highest in the earliest hatches and decreases regularly in the later hatches. The same trend may be noted with respect to the percentage of pausing birds. However, the frequency of pausing and the average duration of pause seem to have no relation to the date of hatch. From the average age at pause onset, which tends to decrease with the date of hatch, it may be seen that in this population the factor bringing on pause operated approximately at the same time. This is borne out by the average date of pause onset, which fell within a period of 14 days in the five hatches studied. Apparently seasonal and age effects are both responsible for the condition of winter pause. Once the birds

pause, however, their age has no effect on the length of time they stay out of production.

Table 2 presents an analysis of variance of degree of pausing between and within the 10-day periods between November 1 and February 28. This analysis, made by pooling the variances for the sire families, indicates significant differences between periods in degree of pausing. Furthermore, the table also shows that there are differences between the families of the different sires and gives the variance between hatches and periods, thus indicating three significant factors in the degree of pausing: (1) Season (between periods), (2) age (between hatches), and (3) inheritance (between sire families). A triple criterion analysis (hatch, period, sire) was not attempted because the numbers in the subclasses are disproportionate, and some of the subclass numbers would be so small as to render the determination of degree of pausing inaccurate.

TABLE 1.—Characteristics of the J series with respect to date of hatch

Date of hatch	Total birds	Proportion of birds pausing	Total pauses	Pauses per pausing bird (frequency)	Average age at pause onset	Average date at pause onset	Average duration of pause	Degree of pausing
	<i>Number</i>	<i>Percent</i>	<i>Number</i>		<i>Days</i>		<i>Days</i>	
Mar. 20.....	237	84.0	323	1.62	257	Dec. 2	28.2	28.6
Mar. 27.....	130	71.5	125	1.34	253	Dec. 5	31.1	23.4
Apr. 3.....	124	62.1	123	1.60	257	Dec. 16	25.1	19.0
Apr. 10.....	193	64.2	180	1.45	240	Dec. 6	24.9	18.9
Apr. 17.....	84	48.8	58	1.41	239	Dec. 12	25.3	13.2
All hatches.....	768	69.5	809	1.51	252	Dec. 6	27.2	21.9

TABLE 2.—Analysis of variance of degree of pausing, J series, between and within 10-day periods from November 1 to February 28

Source of variance	Degrees of freedom	Sum of squares	Mean square
Total.....	2,399	496,339	-----
Between periods.....	11	460,771	41,888
Within periods.....	2,388	35,568	15
Total.....	239	56,546	-----
Between sire families.....	19	10,593	558
Between periods.....	11	35,156	3,196
Remainder.....	209	10,797	52
Total.....	59	9,671	-----
Between hatches.....	4	1,655	414
Between periods.....	11	6,283	571
Remainder.....	44	1,733	39

The trend of degree of pausing by the 10-day periods can be seen from table 3, which gives the distribution by sires and periods. The family showing the highest degree of pausing is that of sire G36 with a degree index of 37.7; the lowest degree observed is 11.8 for sire F15, the other sire families falling within this range. The significant point in this table is that 14 out of the 20 sire families show the mode of pausing in the same period (December 1-10); 4 families have a mode in the neighboring periods. Only 2 of the families show modes in other periods and only 1 of these differs significantly

from the degree of pausing in the December 1-10 period. The significance of differences between any 2 cells may be ascertained from the values given in the footnote to the table. Table 3 thus presents further evidence for the interpretation of table 2 so far as sire family and period differences are concerned.

Table 4 supplies similar evidence with respect to the effect of hatch. The mode for the whole of the population falls in the period of December 1-10, three earlier hatches showing the highest degree of pausing during that period. The two later hatches have modes in the preceding period which, however, are not significantly higher than the degree-of-pausing values for these hatches for the modal period of the whole population.

Degree of pausing was broken down into its constituents: (1) Percent of birds exhibiting pause, (2) frequency or number of pauses per pausing bird, and (3) average duration of each pause. These, together with date of pause onset, were analyzed with respect to differences between hatches and to differences due to variation in the date of first egg. To facilitate the analysis of variance, the periods of maturity here selected (September 18 to November 17) were such as to have representatives of all hatches in each of the four 15-day periods. This included 718 of the 768 birds in the J series.

TABLE 3.—*Distribution of degree of pausing by sires and by periods, J series*¹

Period	Degree of pausing classified by sire and number of daughters as indicated									
	F15, 29	H58, 35	H62, 48	G14, 65	H42, 29	H79, 78	H8, 42	H90, 66	H91, 39	H39, 24
Nov. 1-10.....	20.0	13.9	5.3	5.2	4.8	13.8	2.1	6.0	12.3	0
Nov. 11-20.....	15.4	14.9	13.7	15.9	4.0	15.7	13.5	11.1	22.0	15.3
Nov. 21-30.....	<i>31.8</i>	27.9	27.4	35.5	13.7	29.6	35.9	17.9	39.4	42.9
Dec. 1-10.....	29.2	<i>33.4</i>	<i>31.2</i>	<i>41.5</i>	28.2	<i>33.2</i>	<i>42.7</i>	30.5	<i>51.8</i>	<i>40.5</i>
Dec. 11-20.....	14.7	22.4	19.9	31.2	28.2	28.0	32.4	30.7	39.0	36.0
Dec. 21-30.....	5.0	17.8	14.1	21.3	28.8	25.5	28.1	23.4	24.0	38.1
Dec. 31-Jan. 9.....	12.2	8.7	16.0	19.2	<i>37.9</i>	24.0	27.4	26.3	24.0	31.9
Jan. 10-19.....	8.2	4.2	14.8	13.8	32.1	20.6	22.3	24.5	15.6	30.7
Jan. 20-29.....	2.4	.3	7.2	6.0	21.9	17.0	16.0	21.8	8.9	16.5
Jan. 30-Feb. 8.....	2.1	.9	5.8	1.5	10.5	11.8	10.0	17.9	3.6	2.8
Feb. 9-18.....	0	2.1	5.8	4.5	8.5	9.2	5.7	17.6	5.6	0
Feb. 19-28.....	0	1.2	4.8	3.1	8.7	4.7	5.0	19.7	3.8	0
Total degree of pausing.....	11.8	12.3	13.8	16.6	18.9	19.4	20.1	20.6	20.8	21.2

Period	Degree of pausing classified by sire and number of daughters as indicated									
	G52, 50	G5, 27	H60, 29	H64, 30	H43, 34	H46, 31	H37, 20	H25, 23	H33, 38	G36, 31
Nov. 1-10.....	14.4	4.5	15.8	15.6	9.0	2.1	4.5	19.6	27.2	15.5
Nov. 11-20.....	27.0	26.0	24.1	15.8	10.8	13.0	10.0	20.3	23.7	25.0
Nov. 21-30.....	41.6	46.9	<i>55.6</i>	39.5	32.7	33.8	33.8	55.5	41.5	41.5
Dec. 1-10.....	<i>45.0</i>	58.8	41.0	48.8	<i>58.5</i>	<i>55.0</i>	<i>57.0</i>	<i>59.2</i>	43.2	55.8
Dec. 11-20.....	39.8	40.8	33.1	43.1	43.3	45.2	47.5	47.8	<i>50.0</i>	58.9
Dec. 21-30.....	32.2	30.0	31.2	34.9	32.0	43.2	28.5	50.0	41.3	55.9
Dec. 31-Jan. 9.....	26.0	27.2	27.3	32.8	41.6	44.7	45.0	43.0	43.7	<i>60.9</i>
Jan. 10-19.....	16.4	18.2	17.9	30.8	31.7	30.9	44.5	33.5	39.4	55.8
Jan. 20-29.....	13.2	14.6	9.8	23.0	26.3	21.8	34.0	22.5	31.9	40.1
Jan. 30-Feb. 8.....	9.0	10.1	14.7	20.3	21.5	13.0	20.0	9.3	22.5	22.7
Feb. 9-18.....	4.6	.8	8.9	10.0	9.6	22.4	15.5	15.4	19.2	11.8
Feb. 19-28.....	3.8	0	2.8	5.5	5.1	14.0	11.0	4.1	9.8	8.0
Total degree of pausing.....	22.8	23.2	23.5	26.6	26.8	28.3	28.8	31.4	33.2	37.7

¹ 6.7 constitutes a significant, and 9.2 a highly significant difference between any 2 cells.

Modes in italic figures.

TABLE 4.—*Distribution of degree of pausing with respect to date of hatch in the J series*¹

Period	Data for date of hatch indicated					
	Mar. 20	Mar. 27	Apr. 3	Apr. 10	Apr. 17	All hatches
Nov. 1-10.....	12.2	7.3	8.0	10.1	13.8	10.3
Nov. 11-20.....	16.3	10.9	14.8	20.8	20.6	16.6
Nov. 21-30.....	45.3	33.6	21.9	<i>35.2</i>	<i>24.4</i>	34.4
Dec. 1-10.....	<i>59.2</i>	<i>47.6</i>	<i>30.6</i>	32.4	20.9	<i>41.9</i>
Dec. 11-20.....	50.3	43.4	25.6	23.3	17.7	34.9
Dec. 21-30.....	42.5	33.2	20.1	18.2	18.4	28.4
Dec. 31-Jan. 9.....	36.5	31.0	27.8	24.4	12.8	28.5
Jan. 10-19.....	27.1	30.5	22.8	19.1	12.7	23.4
Jan. 20-29.....	19.9	19.9	16.7	11.6	10.7	16.8
Jan. 30-Feb. 8.....	15.7	10.1	14.3	8.0	2.9	11.5
Feb. 9-18.....	11.2	8.7	14.5	6.0	1.3	9.3
Feb. 19-28.....	7.0	5.1	11.0	5.1	1.7	6.6
Total degree of pausing.....	28.6	23.4	19.0	17.9	13.2	21.9

¹ Modes in italic figures.

Table 5 presents this analysis and indicates that date of first egg has more influence on the date of pause onset and on duration of pause than has date of hatch, while the latter has a greater influence in determining the percentage of birds in a population exhibiting pause. Frequency of pause or occurrence of repetitional pausing by the same bird seems to be independent of either date of hatch or date of first egg.

TABLE 5.—*Analysis of variance of pausing characters in the J series*

Source of variance	Degrees of freedom	Date of pause onset		Percentage of birds pausing		Frequency of pause		Duration of pause	
		Sum of square	Mean square	Sum of square	Mean square	Sum of square	Mean square	Sum of square	Mean square
Total.....	24	3,373	-----	72,106	-----	16.15	-----	1,303	-----
Between hatches.....	4	315	78.8	28,564	¹ 7,141	3.46	0.865	260	65.0
Between dates of first egg.....	4	1,874	² 468.5	7,271	1,818	2.28	.570	708	² 177.0
Remainder.....	16	1,184	74.0	36,721	2,267	10.41	.651	335	20.9

¹ Significant.

² Highly significant.

Although the date of first egg apparently bears no relation to the proportion of birds in the population which pause, this does not hold true within sire families. Thus, the date of first egg of pausing half-sisters is 9.85 days earlier than that of the nonpausing birds in the same family. The *t* value for 19 degrees of freedom is 6.5405, which indicates that this difference is highly significant.

The genetic implications involving the factors discussed were analyzed by means of correlations between the characteristics exhibited by sire families. With 20 sires represented in this population, 18 degrees of freedom for the zero-order correlations were available. Table 6 gives the coefficients of correlation and indicates that:

1. Frequency of pauses is not correlated with any of the other factors studied.

2. Date of pause onset is not correlated with any of the other factors studied.

3. Date of first egg of pausing birds is correlated with the percentage of birds in the sire family which exhibit pause, the correlation coefficient being 0.5127.

4. Percentage of pausing birds in the sire family is not correlated with average duration of pause.

5. The degree of pausing shows the greatest correlation with the percentage of pausing birds (0.7421), followed by the correlation with duration of pause (0.5869).

TABLE 6.—Zero-order correlation coefficients between characteristics of sire families in the J series¹

Character studied	B	C	D	E	F	G
A. Date of first egg of all birds	0.9309	0.3762	0.1355	-0.2852	0.3043	0.0947
B. Date of first egg of pausing birds		.3825	.0302	-.2233	.5127	.2356
C. Date of pause onset			-.2815	.4200	.1935	.2950
D. Frequency of pause				-.3226	-.1733	-.0265
E. Duration of pause					.1476	.5869
F. Percentage of birds pausing						.7421
G. Degree of pausing						

¹ r at $P=0.05$ is 0.444; r at $P=0.01$ is 0.561.

The coefficient of multiple correlation, with degree of pausing as the dependent variable and frequency of pause, duration of pause, and percentage of pausing birds as the independent variables, was found to be 0.9225, giving 85 percent determination. Complete determination of degree of pausing by these three independents is not attained, since the measurement of degree is confined to the period between November 1 and February 28, while some of the pauses started before, and others continued beyond these dates.

TABLE 7.—Characteristics of the K series with respect to date of hatch

Date of hatch	Total birds	Proportion of birds pausing	Total pauses	Pauses per pausing bird (frequency)	Average age at pause onset	Average date at pause onset	Average duration of pause	Degree of pausing
	<i>Number</i>	<i>Percent</i>	<i>Number</i>		<i>Days</i>		<i>Days</i>	
Mar. 19	127	55.1	109	1.56	246	Nov. 20	26.2	11.8
Mar. 26	166	48.8	141	1.74	246	Nov. 27	28.6	13.2
Apr. 2	179	46.4	127	1.53	249	Dec. 7	26.4	12.3
Apr. 9	154	34.4	74	1.40	249	Dec. 14	24.5	8.3
All hatches	626	45.8	451	1.57	248	Dec. 2	26.7	11.4

DATA ON THE K SERIES

The characteristics of the K series of birds are presented in table 7. A comparison of this table with table 1 shows considerable differences in the pausing characters of the two populations. Thus, the degree of pausing in the J series is nearly twice that exhibited by the K pullets. The trend in the latter with respect to date of hatch is not so uniform as that found in the J birds, although the last hatch shows the least amount of pause. The percentage of pausing birds is considerably higher in the J series, while pause frequency and pause duration are not markedly different in the two populations. The most significant

differences between the J and the K series lie in the average age and the average date of pause onset in the different hatches, although when each population is considered irrespective of hatching dates, these differences are not marked. While the pauses in the different hatches of the J series occurred at approximately the same date, the K population showed increasingly later onset of pause with the later date of hatch. As a consequence, in the J series the pausing birds in the late hatches were younger than those in the early hatches at the time of pause onset, whereas in the K series a uniformity in age of pausing birds, irrespective of date of hatch, obtained.

TABLE 8.—*Distribution of degree of pausing with respect to date of hatch in the K series*¹

Period	Data for date of hatch indicated				
	Mar. 19	Mar. 26	Apr. 2	Apr. 9	All hatches
Nov. 1-10.....	10.6	14.3	9.1	4.9	10.0
Nov. 11-20.....	12.9	11.5	13.7	9.6	11.6
Nov. 21-30.....	13.1	13.2	16.7	10.6	13.4
Dec. 1-10.....	10.0	<i>16.3</i>	<i>20.9</i>	<i>13.4</i>	<i>15.4</i>
Dec. 11-20.....	13.4	15.6	19.4	11.5	15.3
Dec. 21-30.....	12.6	9.7	11.7	8.8	10.5
Dec. 31-Jan. 9.....	12.2	12.0	7.7	3.3	8.7
Jan. 10-19.....	<i>15.8</i>	14.1	10.5	7.0	11.7
Jan. 20-29.....	14.6	15.8	11.8	8.6	12.7
Jan. 30-Feb. 8.....	9.0	11.9	10.1	11.2	10.8
Feb. 9-18.....	7.7	12.4	9.3	6.4	9.0
Feb. 19-28.....	9.2	11.7	6.7	4.8	8.6
Total degree of pausing.....	11.8	13.2	12.3	8.3	11.4

¹ Major modes in italic figures.

A further difference in the behavior of the 2 series may be observed from the comparison of tables 4 and 8, which give the distribution of the degree of pausing in the respective series by periods. Whereas the J series in general gives a unimodal type of distribution, the K series invariably shows 2 or more peaks for each hatch. The reasons for these observed differences are somewhat obscure. It is not certain that the reduction in number of pauses (809 pauses in a flock of 768 birds or 1.05 per bird in the J series; 451 pauses in a flock of 626 birds or 0.72 per bird in the K series) did not lead to the differential behavior observed.

TABLE 9.—*Zero-order correlation coefficients between pause characters of sire families in the K series*¹

Character studied	B	C	D	E	F	G
A. Date of first egg of all birds.....	0.8334	0.2920	-0.0997	-0.2726	0.1695	0.1545
B. Date of first egg of pausing birds.....		.2782	-.0754	-.3582	.2491	.1853
C. Date of pause onset.....			.0940	-.3552	.1389	.0929
D. Frequency of pause.....				-.4336	.7492	.6796
E. Duration of pause.....					-.4840	-.1023
F. Percentage of birds pausing.....						.8786
G. Degree of pausing.....						

¹ r at $P=0.05$ is 0.482; r at $P=0.01$ is 0.606.

Some differences may be also noted between the two populations with respect to the magnitude of the correlation coefficients between the different pause characters of sire families in the two populations. Table 9 lists the coefficients for the K population corresponding to those for the J series in table 6. There is agreement between the two tables as far as the majority of the correlation coefficients is concerned. There are, however, a few notable differences. Thus, while frequency of pause shows no correlation with percentage of birds pausing (-0.1733) or with degree of pausing (-0.0265) in the J series, in the K population the respective coefficients are 0.7492 and 0.6796 . This changed relation also brings about differences in the coefficients of correlation between duration of pause and percentage of birds pausing (-0.4840 for the K series as compared to 0.1476 for the J) and between duration of pause and degree of pausing (-0.1023 for the K series and 0.5869 for the J). The coefficient of multiple correlation between degree of pausing on the one hand and frequency of pause, duration of pause, and percentage of birds pausing on the other is somewhat higher in the K series (0.9560) than in the J (0.9225), the former yielding 91 percent determination. Percentage of birds pausing seems to be the factor of major importance in accounting for differences in the degree of pausing in both of the series. This suggests that in breeding against occurrence of winter pausing the percentage of pausing birds, rather than duration or frequency in a family might be used as the standard of selection.

The general picture obtained from analysis of the J series is not particularly changed by the addition of the data for the K population. The fact that some of the relations between pause characters of sire families are different in the two populations argues for the point of view that the relations vary with the degree of pause observed.

The results from repetitional matings in the 2 years for which the data are considered may be of some interest. Table 10 presents the pause characteristics of the progeny of males mated in both years, as well as the performance of full sisters hatched in the 2 years. Only those matings which produced five or more sisters in each of the series are listed. It is apparent from this table that while the degree of pausing is higher in the J series, there is no consistent difference in either frequency or duration of pause between the J and K pullets. The percentage of birds pausing is, however, invariably greater in the J series sires' progeny and in full-sister families. This confirms the interpretation placed on the correlation coefficients. The same is true for all but one of the full-sister families with respect to degree of pausing.

It may be noted that for all of the repetitional matings the incidence of pausing birds is reduced 35.7 percent in the K over the J series. For the whole flock the reduction is 34.1 percent. This suggests that the major factors responsible for a lower percentage of pausing birds in the K series are environmental in nature, rather than genetic.

In absence of controlled experimental results it is not possible to say what these factors may be. However, by comparison of the performance of birds from the repetitional matings presented in table 10 and from other available information, some of the possible reasons for the observed differences in pause behavior may be considered.

TABLE 10.—Pause characteristics of repeat matings

Sire or mating	Series	Total birds	Proportion of birds pausing	Pauses per pausing bird (frequency)	Average duration of pauses	Degree of pausing
		<i>Number</i>	<i>Percent</i>		<i>Days</i>	
G14.....	J	65	66.2	1.33	23.8	16.6
	K	35	37.1	1.38	28.6	6.9
G52.....	J	50	74.0	1.57	24.0	22.8
	K	29	44.8	1.38	28.4	10.8
H33.....	J	38	81.6	1.55	34.4	33.2
	K	32	43.8	1.57	35.7	14.1
H60.....	J	29	82.8	1.83	15.8	23.5
	K	42	78.6	1.67	18.2	18.8
H90.....	J	66	63.0	1.31	35.4	20.6
	K	59	39.0	1.43	23.2	9.0
G14♂F424.....	J	6	33.3	1.50	14.3	5.7
	K	6	16.7	1.00	25.0	17.5
G14♂G738.....	J	9	55.6	1.60	25.5	1.5
	K	5	20.0	2.00	9.0	5.1
G14♂G742.....	J	12	48.3	1.29	12.6	7.3
	K	7	42.9	1.00	44.8	25.4
G52♂H590.....	J	11	81.8	1.56	27.6	8.1
	K	6	66.7	1.75	9.3	12.6
H90♂G312.....	J	7	57.1	1.25	20.4	6.8
	K	6	33.3	1.50	20.7	

As reported elsewhere (9), each of the two series was divided approximately evenly, so that one-half of the pullets of each mating were on one ration, and one-half on another. The difference between these rations was in the percentage of wheat bran in substitution for whole wheat, the percentage of protein and minerals being adjusted by other slight changes. One ration was common to both series. Despite differences in age at sexual maturity between the groups on different rations in the J series, there was no difference in the extent of winter pause. The K series on the whole exhibited earlier sexual maturity than the J series, though no difference was found in age at first egg of pullets on the two rations used in the K series. However, sexual maturity cannot be considered as a differential factor between the two series, since as shown in table 5 it has little bearing on the characters determining the degree of pausing. The difference in maturity between pausing and nonpausing birds within families existing above, if effective between series, would operate in the direction of increasing rather than decreasing the proportion of pausing birds in the K series above that of the J series.

There was no difference in extent or type of chick mortality in the repetitional matings of the two series, but the rate of growth was higher for the pullets of the K series. The average weight at 6 weeks of age in the repetitional matings was nearly 100 grams higher in the K series (342.1 grams against 248.1 grams in the J series). What bearing this observation may have on the nature of winter pausing cannot be determined from the data on hand.

So far as repetitional pausing of the same bird is concerned, there seems to be no consistent or significant difference between the duration of the first pause and that of the second (table 11). This was true for both the J and K series. There seems to be no evidence indicating that the second pause in birds pausing twice differs in any appreciable way from the first pause.

CLIMATOLOGICAL OBSERVATIONS

Seasonal curves for the total degree of pausing between November 1 and February 28 were drawn for each of the series and compared with the curves for precipitation, humidity, and maximum, minimum, and mean daily temperatures. These were drawn from data⁴ recorded on the south bank of the canyon. The poultry plant is located on the north bank. No apparent relation between these climatological observations and the degree of pausing was apparent from these curves. Similarly, no apparent relation of the degree of pausing in the K series to daily hours of sunshine could be noted. The sunshine observations were made on the university campus about half a mile from the poultry plant.⁵ No sunshine data for the year of hatch of the J series were available.

As far as the length of day is concerned, the degree of pausing in the J series varies inversely with the length of day up to the first period in December (December 1-10). In the mid-December period the degree of pausing begins to decrease while the length of day reaches its minimum. In general, the fluctuations in degree of pausing follow the changes in day length in a none-too-regular curvilinear fashion. This does not hold true for the K series, in which it appears that the degree of pausing fluctuates fortuitously as far as the length of day is concerned.

TABLE 11.—Duration of pauses of birds pausing twice

Series	Double-pausing birds	Duration of pause		First pause longer	Second pause longer	Two pauses of equal length
		First	Second			
	Number	Days	Days	Number ¹	Number ¹	Number ¹
J.....	136	23.1	20.2	73	58	5
K.....	55	22.3	24.5	27	26	2
Both.....	191	22.9	21.4	100	84	7

¹ Cases.

DISCUSSION

From evidence presented by Hays (5) and by the present writers, winter pause appears to be a character dependent upon both genetic and environmental conditions for its expression. The environmental agents producing a pause are seasonally determined but do not seem to be directly associated with any seasonal changes in the climatological factors studied. Evidence is available that pullet offspring from the same matings in different years respond differently with respect to pause. Since the genetic constitution of such groups of offspring would be expected to be very similar, the difference in incidence of pause might be presumed to be related to differences in environmental conditions between the years.

However, the intrinsic susceptibility of birds to pause does not seem to be constant. With increasing age at the season of the modal pausing period (effect of hatching date) and with increasing time in production preceding this period, there appears to be induced in the bird a physiological condition causing it to respond more readily to

⁴ Supplied through the courtesy of C. J. Kraebel, of the California Forest Experiment Station.

⁵ The sunshine observations were made available to the writers by Prof. John B. Leighly, of the Department of Geography of the University of California.

the environmental stimuli by pausing. Birds with different genetic constitutions show different responses to the same age, production, and environmental effects.

It is possible to conceive of winter pause as being induced by adverse environmental conditions which overcome the resistance of the bird to such external influences. The threshold of response varies with different birds at the same time and under the same conditions, and within the same bird at different times. Once induced, the pause proceeds for a period of time that does not seem to be related to the incidence of pausing in the flock or to the frequency of pausing in the bird. In the case of repetitional pausing, the course of the second pause does not seem to differ from that of the first pause. The duration of extremely long pauses might conceivably express the action of either repeated or continuous periods in which the effect of the adverse environment was above the threshold of response for the individual bird.

The distribution of pauses by 10-day periods between November and February indicated, in the case of the population with the high degree of pause, a uniform modal pausing period irrespective of the date of hatch. In the case of the population with a low degree of pausing, multimodal distribution prevailed, but the major modes in three out of four hatches fell in the same period as did the modes in the other population.

The seasonal distribution of pausing in the progenies of different sires showed considerably uniformity.

Date of pause onset and duration of pause depended largely on the date of first egg in the population as a whole, though no such relation is observed when sire families are considered.

Percentage of birds pausing depended to the greatest extent on date of hatch.

In birds pausing twice, there was no apparent difference between the duration of the first and of the second pauses.

The variation within the year in climatological factors studied did not seem to bear any direct relation to the degree of pausing in either population.

If the foregoing assumptions are true, the character of winter pause, observable only by its presence or absence, may in fact be genetically or physiologically governed by a wide range of variation in response thresholds. It is not likely that this concept of the basis of production of winter pauses can be proved by study of the production records of birds. Physiological studies identifying the changes in the birds associated with changes in thresholds of response would seem to be a more likely means of solution of the problem.

GENERAL CONCLUSIONS

Degree of winter pausing as defined here (percentage of hen-days from November to February spent in pauses of 7 days' duration or longer) is determined by the percentage of birds pausing in a population, by the frequency of pauses, and by duration of pause.

Percentage of birds pausing is of major influence in determining the degree of pausing in a population.

In a population with 21.9 as the degree of pausing, duration of pause was significantly correlated with degree of pausing, while in a popu-

lation with 11.4 as the degree of pause, frequency of pause was of greater importance than duration of pause.

Percentage of pausing birds in a family is suggested as a standard of selection in breeding against occurrence of winter pause.

Degree of pausing depends also on the genetic constitution of the birds (as shown by variance between sires), on the date of hatch, and on the season.

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THE ALLEGED PROTECTIVE ACTION OF ALFALFA AGAINST THE HEMORRHAGIC SWEETCLOVER DISEASE¹

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INTRODUCTION

Reports of losses among cattle caused by the feeding of poorly cured sweetclover (*Melilotus*) hay appear annually. The disease induced by this fodder, often referred to as "sweetclover disease," is characterized by a diminished clotting power of the blood and, in the advanced stages, by severe hemorrhages which usually lead to the death of the animal. In a recent paper on one aspect of this problem Quick³ concludes:

Diet is the important means for controlling the disease. The incorporation of 5 percent of dehydrated alfalfa meal with the toxic hay was found sufficient to prevent the development of the disease or even any demonstrable reduction of prothrombin. * * * The animal [rabbit] appears to be able to store this accessory factor [from alfalfa], for it is very difficult to produce sweet clover disease in animals that have been fed relatively large amounts of alfalfa. This explains why some animals are far more resistant than others to the same lot of spoiled hay.

Quick points out the practical significance of these conclusions and calls attention to the possible relation of the above-mentioned accessory factor to the antihemorrhagic vitamin (K) required by the chick.⁴ Quick's conclusions, however, are not in accord with the results obtained in the writer's studies of this disease.

VARIATION IN REACTION OF RABBITS TO TOXIC HAY

Schofield,⁵ who earlier had called attention to the causal relation of spoiled sweetclover hay and silage to this disease, observed that young animals are more susceptible to the toxic principle than mature animals. This was apparent in the writer's early tests with rabbits. The studies of Roderick and Schalk⁶ on this disease were made largely on cattle, but their tests on rabbits indicated that "a group of rabbits shows somewhat more variation in the time interval required for the disease to develop with a given hay than in a group of cattle." In the writer's experiments a marked variation in the response of rabbits of

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² The writer is indebted to Dr. R. A. Brink for suggestions and counsel in these studies.

³ QUICK, ARMAND J. THE COAGULATION DEFECT IN SWEET CLOVER DISEASE AND IN THE HEMORRHAGIC CHICK DISEASE OF DIETARY ORIGIN. *Amer. Jour. Physiol.* 118: 260-271, illus. 1937. See pp. 263-264 and 269.

⁴ DAM, HENRIK, and SCHÖNHEYDER, FRITZ. A DEFICIENCY DISEASE IN CHICKS RESEMBLING SCURVY. *Biochem. Jour.* 28: [1355]-1359, illus. 1934.

⁵ SCHOFIELD, FRANK W. DAMAGED SWEETCLOVER: THE CAUSE OF A NEW DISEASE IN CATTLE SIMULATING HEMORRHAGIC SEPTICEMIA AND BLACKLEG. *Jour. Amer. Vet. Med. Assoc.* (n. s. 17) 64: 553-575. 1924.

⁶ RODERICK, LEE M., and SCHALK, A. F. STUDIES ON SWEETCLOVER DISEASE. *N. Dak. Agr. Expt. Sta. Bull.* 250, 56 pp., illus. 1931. See p. 10.

similar age to a given toxic hay has been noted. Moreover, animals found susceptible in a preliminary test gave a similar reaction in subsequent exposures to the toxic principle, allowance being made for advancing age. The degree of toxicity of 15 samples of poisonous hay has been determined by pairs of rabbits found to be susceptible in a preliminary test, and in no instance has a significant difference been observed in the reaction of the two animals to any one hay. Furthermore, some rabbits have been included in as many as 10 trials with no apparent change in susceptibility other than that associated with advancing age. Some 150 rabbits have been used in these experiments.

Very few tests have been made on resistant animals, but one example may be cited. Six rabbits 6 weeks old were obtained from a local rabbitry in which no alfalfa was being fed. Toxic sweetclover hay was introduced gradually into the diet. For the first 2 days, the diet contained 15 percent of toxic hay, for the third day 40 percent, and for the fourth and fifth days 90 percent, the remainder of the diet in each instance being ground oats. Each rabbit received the oats and hay mixture in daily amounts of 45 gm. per kilogram of body weight. On the sixth day the clotting time of the blood of each rabbit was determined.⁷ Of the six rabbits, three showed a blood-clotting time of over 60 minutes as compared with the normal 5 to 7 minutes; one, S6, showed a clotting time of 50 minutes; and two, S2 and S3, showed respectively 8 and 6 minutes. The four susceptible rabbits were transferred to nontoxic feed, while S2 and S3 were continued on the diet containing 90 percent of toxic hay for a further period of 7 days. During this period five tests of the clotting time of the blood were made, the highest value for S2 being 10 minutes and for S3 9.5 minutes, indicating a high resistance to the action of the toxic principle. After an interval of 86 days, during which S2 had been fed timothy hay and a mixed grain while S6 had been used in two tests for the toxicity of spoiled nonbitter sweetclover, the two rabbits were given an exclusive diet of toxic hay in daily amounts proportional to body weight. The data from this test shown in table 1 demonstrate that S2 maintained its resistance over an 86-day period on a diet devoid of alfalfa.

TABLE 1.—Reaction of 2 rabbits, 1 resistant and 1 susceptible, when 6 weeks old, to an exclusive diet of toxic hay begun when rabbits were 20 weeks of age¹

Rabbit No.	Reaction when 6 weeks old	Blood coagulation of rabbit	
		Period on diet	Coagulation time
		Days	Minutes
S2-----	Resistant-----	0	6
		5	6
		10	5
		18	6
S6-----	Susceptible-----	0	5½
		2 5	25

¹ In the interval between the 6-week and 20-week tests, the diet of S2 was timothy hay and mixed grain; that of S6 after 5 days on toxic diet was timothy and mixed grain for 14 days, spoiled *Melilotus dentata* hay for 63 days, and timothy and mixed grain for 16 days.

² Ear hemorrhage from incision on fifth day; transferred to nontoxic diet.

⁷ The determination was made as follows: Approximately one-half cubic centimeter of blood was allowed to drop from a freely bleeding incision in the marginal vein of the ear into a 50-mm. watch glass. Another watch glass was inverted over it, and the time required for the formation of a firm clot was determined at room temperature (23°-24° C.) by tilting the watch glasses at 30-second intervals.

The above data suggest that the variation in a group of rabbits in their reaction to a given toxic hay is due to the inherent characteristics of the animals and is not the result of previous feeding. No attempt has been made to test this interpretation further by the mating of resistant with resistant animals and susceptible with susceptible. In assembling a stock of rabbits for critical comparisons of the toxicity of various spoiled hays and extracts, the writer has adopted the procedure of subjecting a group of rabbits of similar age to a preliminary test on a known toxic hay. The animals showing a prompt response to the toxic principle are transferred to nontoxic feed in an early stage of the disease. The clotting time of the blood of these rabbits is normal when tested after an interval of 10 days and the animals are used in further experiments. In preliminary tests it became apparent that unless this variable of rabbit reaction is recognized and under control, an experiment involving a small number of unselected animals may be misleading.

ADDITION OF ALFALFA TO A TOXIC DIET

The writer has found no evidence in favor of the protective effect of small amounts of alfalfa. Roderick and Schalk⁸ tested various vitamins and feeds, including alfalfa, on cattle with negative results. The writer has fed alfalfa up to 50 percent of the diet along with toxic hay and has observed the symptoms of the disease as determined by blood-coagulation tests. Moreover, a majority of the animals used in these experiments have received alfalfa *ad libitum* along with a grain mixture from the time of weaning until tested for reaction to toxic hay. No difficulty has been encountered in finding animals that react readily, and no significant differences in susceptibility have been found between groups of animals that received no alfalfa in the period prior to the preliminary test and groups that received alfalfa. Some experiments, however, have been made to test more fully the relation of alfalfa to the incidence of the disease.

In the first test a choice sample of commercial alfalfa hay, grading U. S. No. 1, Extra Leafy Extra Green was compared with a mixed grain for protective activity when fed as a supplement to a toxic sweetclover hay. Four rabbits that had been susceptible in early preliminary tests were first fed for 5 days on a known toxic hay in daily amounts proportional to body weight. The clotting power of the blood was then determined. After an interval of 9 days on timothy and mixed grain, two were put on a diet consisting of 10 percent of mixed grain and 90 percent of toxic hay and the other two on 10 percent of alfalfa and 90 percent of toxic hay. The results are summarized in table 2.

As will be noted in table 2, the clotting time of the blood from the rabbits when receiving supplement was determined after 5 days of feeding. Difficulty was encountered in arresting hemorrhage from the incision in the marginal ear vein in rabbits 2 and 4. To permit the disease to run a natural course, no further blood samples were taken and the ration was fed until the death of each animal. All four rabbits succumbed after a short feeding period and all showed hemorrhagic lesions typical of the disease. No significant difference in

⁸ RODERICK, L. M., and SCHALK, A. F. See footnote 6

reaction was detected between the two pairs of rabbits and there was no suggestion of any protection resulting from the incorporation of 10 percent of a high-grade commercial alfalfa in the toxic diet.

TABLE 2.—*Effect of feeding susceptible rabbits toxic sweetclover hay, with and without supplement, as measured by the clotting power of their blood after 5 days of feeding*

Rabbit No.	Blood coagulation of rabbits fed—				
	Toxic hay		Toxic hay+supplement		
	Period on diet	Coagulation time	Supplement (10 percent of diet)	Period on diet	Coagulation time
	Days	Minutes		Days	Minutes
1.....	0	6½	Mixed grain.....	0	5½
	5	14	15	13
2.....	0	5½	Mixed grain.....	0	5½
	5	12	25	23
3.....	0	4½	Alfalfa.....	0	6
	5	17	25	15
4.....	0	4½	Alfalfa.....	0	5
	5	12	15	16

¹ Died of internal hemorrhages on 9th day.

² Died of internal hemorrhages on 13th day. Rabbit No. 2 consumed only small amounts of the ration from the 6th to the 10th day.

³ Died of internal hemorrhages on 7th day.

One might, however, postulate that freshly cut alfalfa contains a protective factor which was inactivated during the curing or storage of the sample used in the foregoing experiment but preserved unimpaired in the alfalfa meal used in the experiment of Quick.⁹ To obtain information on this point an experiment was made in which freshly cut alfalfa was used as a supplement to toxic sweetclover hay.

A 40-gm. lot of a poisonous sweetclover hay was fed to a susceptible rabbit. After an interval of 39 hours from the time of feeding, the prothrombin content of the blood was determined¹⁰ by a modification of the method of Quick, Stanley-Brown, and Bancroft.¹¹ The prothrombin activity was found to have been reduced to 38 percent of normal. After a 21-day interval during which the prothrombin content returned to normal as determined by the above method, the animal again received 40 gm. of the same toxic forage and in addition 20 gm. of freshly cut alfalfa, which on a dry-weight basis constituted 12.9 percent of the ration. A test after 36 hours showed a prothrombin activity 42 percent of normal. In view of the regular response of susceptible animals to repeated treatments with the same toxic hay, it is apparent that the freshly cut alfalfa in the diet did not protect the animal against the action of the toxic principle.

Considerable interest has recently been shown in the artificial drying of legumes and grasses as a means of obtaining a product rich in accessory nutritional factors. A test similar to the one just described was made by using artificially dried alfalfa instead of the fresh forage.

Forty grams of toxic sweetclover hay along with 4.4 gm. (10 percent of the ration) of alfalfa dried for 3 minutes at 350° F. reduced the prothrombin activity in the blood of a susceptible rabbit to 45 per-

⁹ QUICK, A. J. See footnote 3.

¹⁰ The writer is indebted to H. A. Campbell, of the biochemistry research laboratory, for his cooperation in this and the following experiment.

¹¹ QUICK, A. J., STANLEY-BROWN, M., and BANCROFT, F. W. A STUDY OF THE COAGULATION DEFECT IN HEMOPHILIA AND IN JAUNDICE. Amer. Jour. Med. Sci. 190: 269-281. 1935.

cent; 40 gm. of the same hay with the same rabbit in the absence of a supplement gave a prothrombin reading of 48 percent. Obviously this alfalfa was ineffective in preventing the development of the disease.

SUMMARY AND CONCLUSIONS

Marked differences in the susceptibility of rabbits of similar age to the sweetclover disease have been observed in the course of experiments involving the assay of toxicity of various spoiled sweetclover hays. Animals shown to be susceptible in a preliminary test gave a similar reaction in subsequent tests although becoming progressively less susceptible with advancing age. Resistant animals, insofar as they have been tested, have maintained their resistance. It is suggested that this variation in a group of rabbits is an expression of the inherent constitution of the animals; there is no evidence in these experiments that the differences in the reaction of rabbits to a given toxic hay are induced by previous diet.

Experiments have been performed to investigate Quick's conclusion that alfalfa contains an accessory factor protecting animals against the sweetclover disease. A supplement of commercial alfalfa hay of excellent quality was fed to the amount of 10 percent of the ration along with a known toxic hay and compared with a supplement of mixed grain fed in like amount. Neither supplement gave any indication of retarding the progress of the disease.

Likewise freshly cut alfalfa to the amount of 12.9 percent of the ration of one susceptible rabbit and kiln-dried alfalfa as 10 percent of the diet of another showed no protective activity.

While Quick concludes that 5 percent of alfalfa in a diet of toxic sweetclover hay prevents the development of any symptoms of toxicity, the writer's results indicate that even 12 percent has no significant effect in checking the onset and fatal termination of the disease.

Because of these negative results in the control of the disease by alfalfa in rabbits, it would appear unsafe to attempt the control of sweetclover disease in livestock by the inclusion of a small quantity of alfalfa hay in a diet of sweetclover hay.

RELATIONSHIPS BETWEEN RAINFALL AND COFFEE YIELDS IN THE KONA DISTRICT, HAWAII¹

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INTRODUCTION

The production of coffee in the Hawaiian Islands was begun more than a hundred years ago, although exports were not recorded until 1845. Since the turn of the century over 90 percent of the production has been centered in the Kona district on the island of Hawaii.

An inspection of crop census reports reveals large annual fluctuations in the amount of coffee produced in Hawaii. Controlled measurements made in conjunction with coffee fertilizer experiments have shown fluctuations in yield amounting to over 100 percent, suggesting some dominant climatic or growth factors. In the present paper it will be shown, by applying modern statistical methods of analysis to small samples of data, that coffee production in Hawaii is highly correlated with rainfall

THE KONA DISTRICT

The Kona district is a strip of terrain along the lee or southwest coast of the island of Hawaii, rising in a fairly uniform slope from sea level to 3,500 feet in approximately 5 miles. The coffee-producing area is a narrow belt about 22 miles long and 1 mile wide, ranging from 800 to 2,200 feet in elevation and consisting of small, fairly fertile pockets between relatively recent lava flows. At present, approximately 5,000 acres are devoted to the growing of coffee. The geological and geographical features of this area and its agriculture have been described by Powers, Ripperton, and Goto.²

The seasonal production of coffee in the Hawaiian Islands from 1900 to 1936 has been estimated by Cady, Maneki, and Murata.³ The data from 1900 to 1920 were based on exports; those from 1921 to 1936 were based on the crop census reports of the Agricultural Extension Service, University of Hawaii. These data are recognized not to be precise estimates for the Kona district, as during the period not exceeding 5 percent of the total volume of coffee produced in the Hawaiian Islands was in districts other than Kona. Allowing for fluctuations in yield, there has been since 1900 a steady increase in the production of coffee in Kona, which may be attributed to the following factors: (1) Increased acreage, (2) increased age of the trees, and (3) improvements in fertilizer and cultural practices.

¹ Received for publication November 18, 1938. This investigation was supported in part by a special grant from the Territorial Legislature for coffee investigations in the Kona district.

² POWERS, H. A., RIPPERTON, J. C., and GOTO, Y. R. SURVEY OF THE PHYSICAL FEATURES THAT AFFECT THE AGRICULTURE OF KONA DISTRICT OF HAWAII. Hawaii Agr. Expt. Sta. Bull. 66, 29 pp., illus. 1932.

³ CADY, H. B., MANEKI, M., and MURATA, K. COFFEE PRODUCTION IN HAWAII: A FIVE-YEAR SUMMARY OF COST AND EFFICIENCY STUDIES. Hawaii Univ., Agr. Ext. Serv. Cir. 32, 44 pp., illus. 1937. [Mimeographed.]

In the Kona district, coffee cherries mature and are harvested, for the most part, during the months from August through December. The blossoming season is in the early spring, usually from January through March. Each tree has two or more distinct periods of flowering, depending, supposedly, upon meteorological conditions.

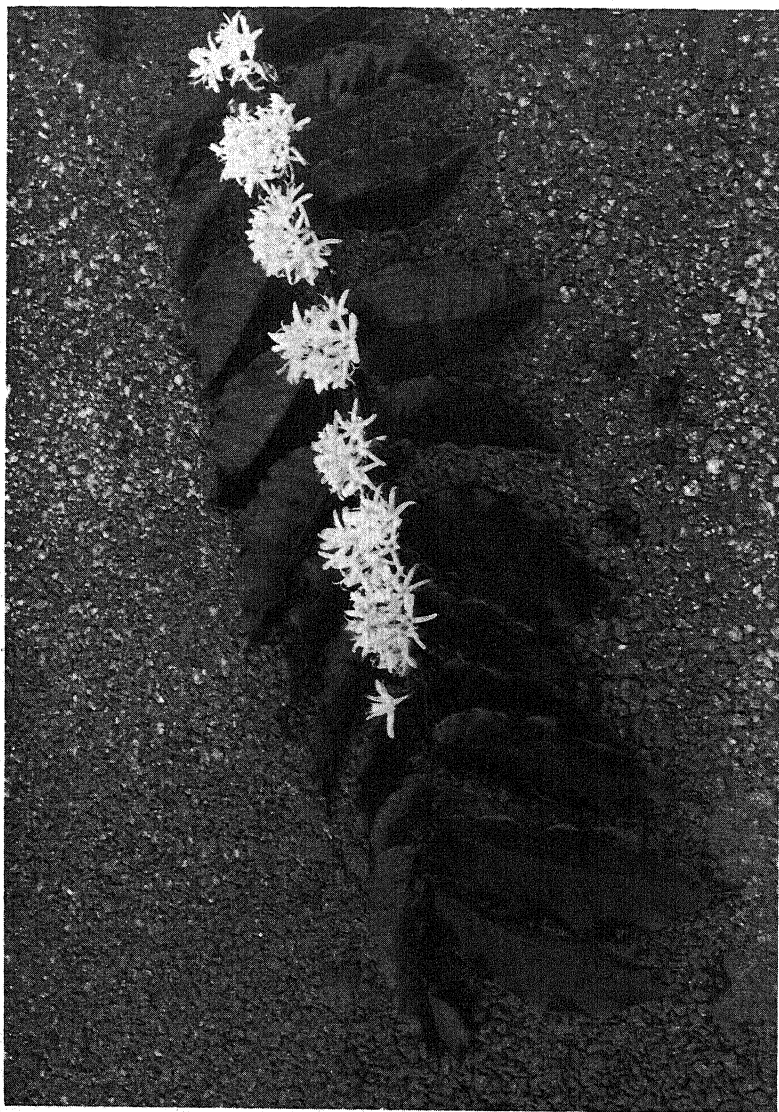


FIGURE 1.—Primary lateral of a coffee tree in early spring showing simultaneous blossoming and spring growth.

During the spring, for 6 weeks to 2 months simultaneously with and following the flowering, occurs the maximum growth of laterals (wood which will blossom and bear fruit the following year). Thus the coffee tree is preparing for the next year's crop at the same time that it sets and develops the current year's fruit. This is illustrated

in figure 1, which shows a lateral with flowers and a flush of new growth.

RAINFALL DATA

Rainfall records at Kealakekua, which is fairly close to the center of the coffee area, are available for the years since 1891. Unfortunately, records for one station, at an elevation of 1,580 feet, were kept only until 1914, and those of a second station (elevation of 1,450 feet) which were available from 1901 proved not to be analogous during the overlapping 14-year period. For this reason only the second set of data is used in this study.⁴

The annual fluctuations in rainfall during the period 1901 to 1936 were leveled somewhat by obtaining running 5-year means, as

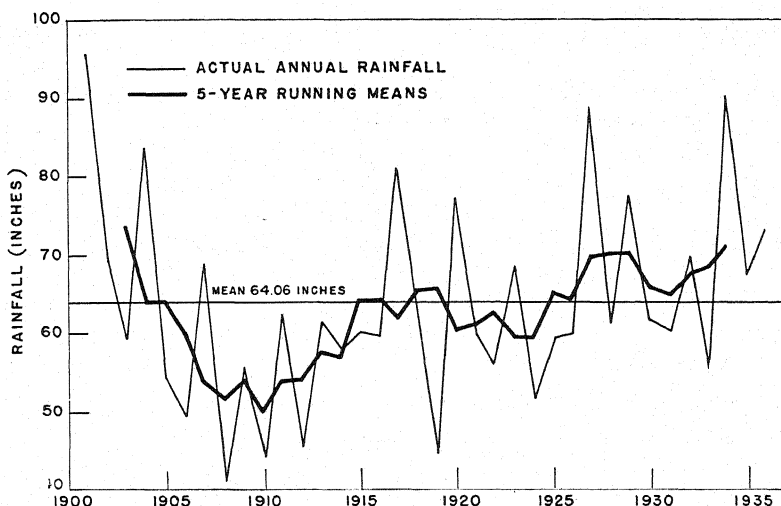


FIGURE 2.—Actual annual rainfall and 5-year running means of annual rainfall at Kealakekua, Kona, Hawaii.

charted in figure 2. It is evident that precipitation was distinctly heavy up to 1905 and from 1925 to 1936; from 1905 to 1915 rainfall was very light, and from 1920 to 1925 it was slightly subnormal.

The monthly distribution of rainfall expressed as means of a 36-year period and the standard errors of these means are given in the following tabulation:

Month:	Mean rainfall (inches)	Month:	Mean rainfall (inches)
January.....	3.68±2.83	July.....	³ 6.88±2.30
February.....	3.28±3.98	August.....	³ 6.99±2.15
March.....	4.02±2.40	September.....	¹ 7.48±3.25
April.....	4.92±3.16	October.....	6.06±3.22
May.....	¹ 6.48±2.82	November.....	¹ 3.71±1.74
June.....	² 6.67±2.69	December.....	3.88±3.73

¹ P value = 0.05-0.02; ² P value = 0.02-0.01; ³ P value = <0.01.

These data show that the so-called rainy season in Kona is during the summer months; this is just contrary to what is common in most

⁴ The rainfall records used in these investigations were obtained from the following: UNITED STATES WEATHER BUREAU. CLIMATOLOGICAL DATA. Hawaii Section.

districts of the Hawaiian Islands. It may also be noted that the months of high rainfall usually have significant means while those of low rainfall have not; in other words, the months having low mean rainfall have the most irregular rainfall.

EFFECT OF RAINFALL ON COFFEE YIELDS

The relationships between rainfall and yield of coffee (*Coffea arabica* L.) in Kona were studied by calculating partial regression coefficients and their sampling errors. The statistical methods used were those described by Fisher.⁵ All regression equations included a term for time in years in order to eliminate the effects of an assumed linear increase in annual production resulting from increased acreage and age of trees and improved cultural practices.

At the outset it seemed desirable to determine whether rainfall directly affected current yield, or indirectly affected yield through the growth of laterals (fruiting wood). For this purpose the following three regression equations were used:

$$(y - \bar{y}) = b_t(t - \bar{t}) + b_{r_1}(r_1 - \bar{r}_1) \quad (1)$$

$$(y - \bar{y}) = b_t(t - \bar{t}) + b_{r_2}(r_2 - \bar{r}_2) \quad (2)$$

$$(y - \bar{y}) = b_t(t - \bar{t}) + b_{r_3}(r_3 - \bar{r}_3) \quad (3)$$

where y = coffee production in million pounds per annum; t = time in years; r_1 = annual rainfall occurring during the years of fruiting; r_2 = annual rainfall occurring during the years of producing fruiting wood; and r_3 = annual rainfall occurring during the years previous to the production of fruiting wood.

TABLE 1.—Partial regression coefficients of coffee production on rainfall occurring during different years of the growth of coffee

Equation No.	Annual (January to December) rainfall occurring during—	Partial regression coefficient
1.....	Years of fruiting (r_1).....	0.0025 ± 0.0176
2.....	Years of producing fruiting wood (r_2).....	1.0378 ± .0161
3.....	Years previous to years of producing fruiting wood (r_3).....	.0062 ± .0153

¹ P value = 0.05 to 0.02.

The partial regression coefficients calculated for the above equations and the standard errors of the coefficients are given in table 1. They show that the rainfall occurring during the year in which new fruiting wood is being produced is significantly related to the succeeding year's yield of coffee. In other words, the rainfall that is related to the seasonal fluctuations in coffee yield does not occur in the year of blossoming, maturing, and harvesting of the cherries. Having established that the rainfall occurring during the preceding crop year is related to the yield of coffee, the annual rainfall was divided into the

⁵ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London, 1934.

following three periods: e , Early rainfall from February to June; m , middle rainfall from June to October; and l , late rainfall from October to February. The relationships between each of these three periods of rainfall and the following year's yield of coffee were studied, the following regression equations being used:

$$(y - \bar{y}) = b_t(t - \bar{t}) + b_e(e - \bar{e}) \quad (4)$$

$$(y - \bar{y}) = b_t(t - \bar{t}) + b_m(m - \bar{m}) \quad (5)$$

$$(y - \bar{y}) = b_t(t - \bar{t}) + b_l(l - \bar{l}) \quad (6)$$

$$(y - \bar{y}) = b_t(t - \bar{t}) + b_e(e - \bar{e}) + b_m(m - \bar{m}) + b_l(l - \bar{l}) \quad (7)$$

The partial regression coefficients and their standard errors calculated for the above equations are given in table 2. It may be seen that

TABLE 2.—*Partial regression coefficients of coffee production on three seasons of rainfall in Kona*

Equation No.	Partial regression coefficients b on—				Standard error of partial regression coefficients of production (y) on—			
	Time	Early rainfall	Middle rainfall	Late rainfall			m	l
	t	e	m	l				
4.....	0.285	0.080			0.0179	0.0228		
5.....	0.279		0.024		.0211		0.0306	
6.....	0.277			0.045	.0505			0.0348
7.....	0.281	0.075	.0064	.0271	.0187	.0239	.0272	.0314

¹ P value = <0.01.

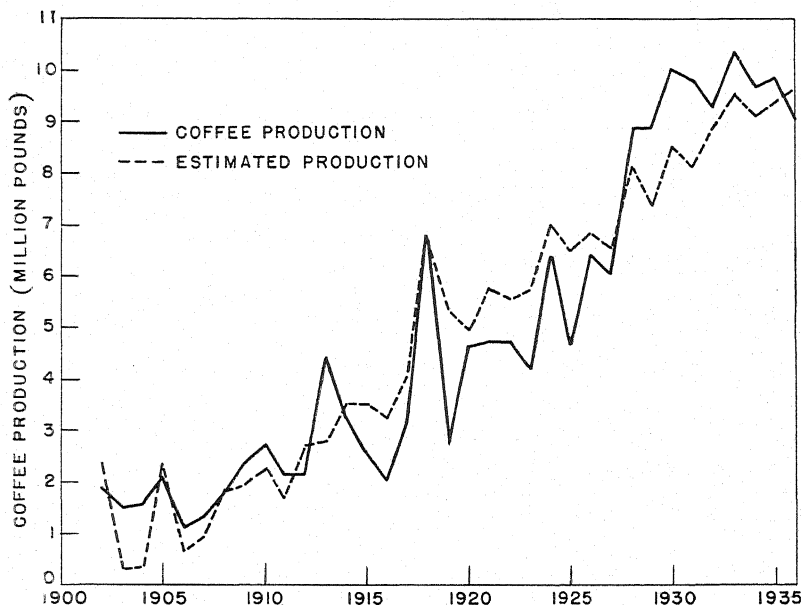


FIGURE 3.—Comparison of the actual coffee production of Kona with that estimated from the equation $y = 0.285t + 0.08e - 1.67$ (where y = estimated production, t = time, and e = February to May rainfall).

only the rainfall occurring between February and June of the preceding season is significantly related to the annual yield. Substituting the calculated values in equation (4), we have

$$y = 0.285t + 0.08e - 1.67$$

This equation was used in obtaining the estimated production of coffee given in figure 3. From the statistical significance of the partial regression coefficient of yield on early rainfall and the general agreement between actual and estimated productions, a considerable amount of the seasonal fluctuation in Kona coffee production may be ascribed to varying early rainfall.

Annual yields of approximately half an acre of coffee trees measured in conjunction with a fertilizer experiment are available for 8 years. Figure 4 shows the annual fluctuations in yield as compared with

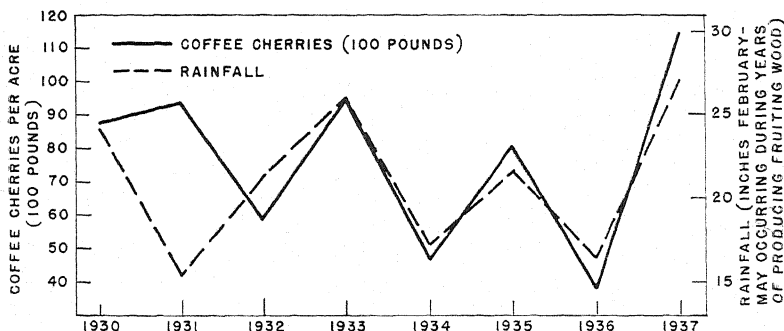


FIGURE 4.—Seasonal fluctuations in yields of a coffee fertilizer experiment, and February to May rainfall occurring during the years of growing fruiting wood.

annual fluctuations in early rainfall occurring during the years of growth of the fruiting wood. It may be seen that, with the exception of 1 year, the fluctuations are concordant. Because of the limited number of observations, statistical methods were not applied to these data.

SUMMARY

Statistical analyses of data on rainfall and coffee production for the years 1901 to 1936 in the Kona district of Hawaii show two distinct periods of heavy rainfall and one period of markedly light precipitation.

The dry season occurs during the winter months, and the months that have low mean rainfall have the most irregular rainfall.

Much of the variability in annual coffee production may be ascribed to fluctuations in the February to June rainfall occurring during the years in which the fruiting wood was produced.

AN ANALYSIS OF GROWTH AND YIELD RELATIONSHIPS OF COFFEETREES IN THE KONA DISTRICT, HAWAII ¹

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INTRODUCTION

The cultivation of coffee (*Coffea arabica* L.) in Hawaii, in view of present world coffee economics, must undergo radical constructive changes if the industry is to attain economic stability. Cultural practices are haphazard and are not based upon practical or scientific principles. Fertilization may range from none to 2,000 or more pounds per acre. In many cases the composition of the fertilizer is not known, and usually applications are not made with consideration of the number of trees per acre, of size and vigor of trees, of previous or present crop, or of rainfall and other weather factors. Pruning may vary from none through that accomplished by the cane knife or machete to the extremely severe, detailed pruning periodically practiced with "topped" coffee.

Nevertheless, average annual yields per acre in Kona are high as compared with those of other countries, and a definite possibility exists that improved practices, based on careful analyses of tree requirements, responses, and yields in relation to the soils and climate of the district, would not only improve the quality and yield but also effect certain economies. The studies here reported were undertaken to secure precise information on the growth responses and yield of the coffee plant in relation to its environment and to serve as a basis upon which to construct more logical and profitable fertilization, pruning, and cultural practices for the district.

REVIEW OF LITERATURE

Growth and fruiting relationships of a number of economic tree fruits, particularly those of temperate-zone regions, have been given careful and detailed study. Trunk-circumference increase, length of terminal growth, length and diameter of fruit spurs, and other measurements have been employed by numerous investigators (Waring (13),³ Collison and Harlan (3), Hofmann (8), Overholser, Overley, and Barnhill (10), and many others). In general, circumference increase and terminal elongation in one growing season have been found to be correlated with the yield in the following year. In fact, it may be said that soil management, fertilization, pruning, thinning, and other important and expensive orchard operations are employed with consideration of their effects upon the crop of the following season as well as their effects upon the volume and quality of the immediate crop. Such studies and applications have not, so far as

¹ Received for publication November 18, 1938.

² The author is indebted to Dr. Lyman A. Dean for aid in securing certain of the growth records and for many helpful suggestions relative to the statistical treatment of the data and the preparation of the manuscript. Credit is due Ah Sin Char for her conscientious work in calculating and checking the statistics.

³ Italic numbers in parentheses refer to Literature Cited, p. 235.

the writer is aware, been extended to the coffeetree nor to many other tropical fruit crops of commercial importance in Hawaii.

The effects of various climatic factors such as rain, frost, wind, etc., on fruit setting and biennial bearing are also well recognized with temperate-zone fruits. Auchter and Schrader (2), Hedrick (7), and Potter (11) have shown that frosts may be the causal factor in bringing about a biennial bearing condition of apple trees. Dean (4) has shown that spring rainfall is a powerful agent in determining annual variations in coffee yields in the Kona district of Hawaii. De Haan (6), who, however, presents very little quantitative data, discusses the effects of rain on flowering and fruit setting. While *Coffea arabica* may bloom at three or more successive periods, the peak of any bloom lasts only 1 day; thus it is entirely possible that certain climatic factors may be operative in Kona, as elsewhere, that induce the extreme annual fluctuations in yield reported by Dean (4) and shown in data reported by McClelland (9).

The literature on pruning and culture of coffee is extensive, but quantitative data on yields, quality, and grade of fruit are lacking except for those of McClelland (9), and perhaps others that have not come to the writer's attention. Alvarado (1) describes pruning systems developed and used in many countries, but again quantitative data on annual yields, costs of pruning and of harvesting, and quality of crop are not stressed. While as yet untested, the more important practices developed elsewhere, applied with a knowledge of climatic effects, tree growth, and fruiting behavior in the Kona district, may be expected to do much to increase annual yields and thus reduce cost of production.

GENERAL DESCRIPTION OF GROWTH HABIT AND MANAGEMENT OF COFFEETREES

The coffee plant, which is a small tree or shrub, has a distinctly dimorphic branching habit, as illustrated in figure 1. The vertical or main stem, as it elongates, produces opposite leaves at each node and a primary lateral shoot in the axil of each leaf. This lateral in turn produces opposite leaves and assumes a horizontal position. If the vertical is bent out at an angle from the axis of the tree, the terminal bud resumes growth in a vertical direction. To bend out a vertical or to cut it back will stimulate the growth of new vertical shoots along the stem immediately below the points of origin of the lateral branch (fig. 1).

Elongational growth of the lateral branch occurs through extension of the apical growing point and also through the formation of branches (secondary laterals) from vegetative buds occurring in the axils of the leaves. A cluster of one to four flower buds is also produced in the axil of each leaf, and each inflorescence produces one to four or more flowers, most of which set fruit. Flowering occurs during the spring rains at two or more periods, on new lateral growth only, produced the preceding growing season. Young trees or 3- to 4-year-old individual verticals of an older tree produce the greater portion of their crop on primary lateral branches; as the tree or vertical becomes older, increasing proportions of the crop are borne on secondary and tertiary lateral branches. In old trees it is impractical to distinguish between primary, secondary, or tertiary lateral growth in the main body of the tree as all are more or less comparable in growth and yield, while the

new primary laterals produced as the vertical elongates make up only a small proportion of the total bearing area of the tree.

The dominant system of training, particularly at medium and lower elevations in the Kona district, is to cut back or to bend over the

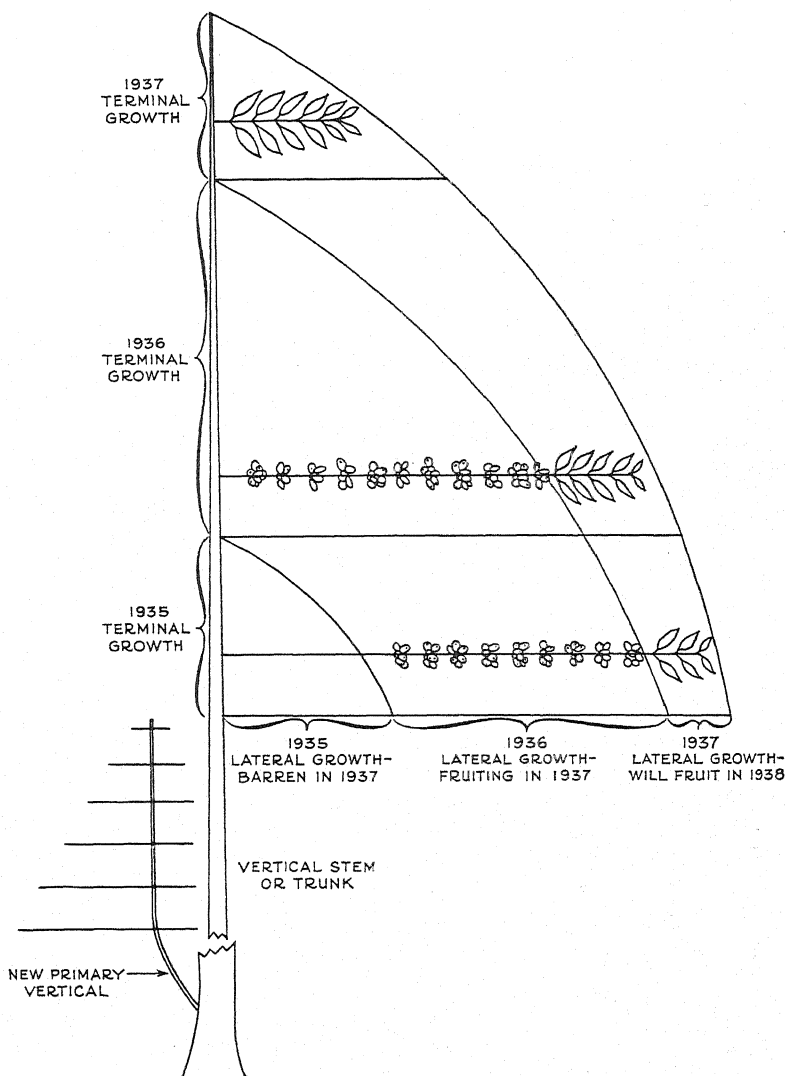


FIGURE 1.—Diagrammatic illustration of the growth and fruiting habit of the coffeetree. Normally two to four, or even more, verticals are trained to form the tree, in which case the primary and secondary lateral branches may overlap. The diagram is drawn to illustrate how biennial bearing may occur. Note the origin of a new vertical shoot. Normally this would not occur unless the old vertical were ringed, bent over, or cut off.

young (1- or 2-year-old) plant, thus stimulating the production of two, three, or even more verticals and training them into the bearing tree. Such a tree may be termed a multiple vertical and is the type used in

this study. Thereafter, little or no pruning is practiced, except removal of any superfluous new vertical shoots, until the tree attains an age and condition when it seems advisable to renew part of the tree by cutting out one of the verticals and training a new one to take its place. Thus, after the orchard has attained an age of 7 years or more, the trees become much less uniform so far as number and age of individual verticals per tree are concerned. No pruning, such as thinning out or heading back of the lateral growth of the tree, is normally practiced with multiple-vertical trees in the Kona district. Occasionally when trees become weakened through overcropping, lack of fertilizer, drought, or a combination of these, heavy heading back of the lateral branches similar to "parrot sticking" may be practiced.

A second general type of training is that of topping. This system consists of cutting off the vertical at a height of 5 to 7 feet above ground and consistently removing all vertical suckers so that the tree never attains a greater height. Growth and fruiting are confined to secondary and tertiary laterals. About once in 4 years the lateral growth is severely cut back with almost a complete loss of crop but with the development of strong new growth.

YIELD AND GROWTH DATA

The data to be used in an analysis of yield and growth relationships are obviously of the utmost importance. Unfortunately, the literature on coffee offered only suggestions as to the more responsive parts of the tree, measurements of which may be significantly correlated with yield, although growth studies of deciduous fruits were helpful. It was necessary to make numerous observations of the growth and cropping behavior of trees throughout the district and finally to take a number of measurements on a restricted group for the purpose of ascertaining their relative applicability and usefulness. Many measurements were discarded after inspection of the data and consideration of the ease and accuracy with which they could be duplicated. Others were not used because they were obviously highly correlated with other measurements (as, for example, the length of terminal growth and the length of primary lateral growth arising from the terminal growth) and consequently would not add materially to the total sum of information.

The coffeetrees used in this study were selected from two experiments: one established on the Fukuda farm, which was designed and installed by J. C. Ripperton, agronomist of the Hawaii Agricultural Experiment Station, to whom great credit is due for his foresight and early work with coffee (12); the other on the Akamatsu farm, which is supported in part by a special grant from the Territorial legislature for coffee investigations in the Kona district.

The preliminary measurements were made on the Fukuda trees; the Akamatsu trees were later selected for study. The following measurements, which are briefly described, were used in the analyses.

Yield: Individual-tree yields were secured and recorded in pounds and tenth-pounds; from four to six pickings over a period of 4 months were required. Only one annual yield (1937) was available for the Akamatsu trees; records for 2 years (1936 and 1937) were used for the Fukuda trees. Weights were obtained with a high-gradespring balance.

Average terminal growth: With the Akamatsu trees the 1936 average terminal growth was estimated by observing from the ground the

terminal growth of each vertical and using as a standard of comparison the 1-foot and $\frac{1}{4}$ -foot divisions of a 12-foot pole employed in measuring tree height. With the Fukuda trees the 1936 and 1937 terminal growth of each fruiting vertical was measured with a flexible tape. Only verticals over 3 years of age were considered as fruiting verticals. The 2 years' terminal growth records were obtained in the same year (at the end of the 1937 growing season), as growth made in each year may be readily distinguished.

Lateral growth: On both the Akamatsu and Fukuda trees, the elongation of the fruiting laterals was obtained at the beginning of the 1938 growing season by measuring and averaging five or more apparently typical, readily accessible lateral growths borne in the larger fruiting area of the tree, at an elevation of 5 to 7 feet. Lateral growths arising from 2- and 3-year-old verticals that were not typical of the greater bearing area were avoided.

Total area of cross section of verticals: The sum of the squared circumferences of the several verticals of each tree was used in the statistical analyses as a means of expressing cross-sectional area. When area is specifically used in the text the appropriate correction factor ($\frac{1}{4}\pi$) was applied. The circumference of the vertical was obtained midway between the first and second nodes above the point of origin of the vertical. When the original vertical was intact it was measured at a point about 8 inches above the highest main vertical arising from it; in case no second vertical was present, the original vertical was measured at a smooth area on the trunk approximately 2 feet above the ground.

Statistical methods: The relationships between growth and yield of the coffeetrees at each of the two locations were estimated from the means and the simple and partial regression coefficients of the measurements. Sampling errors, calculated according to methods given by Fisher (5), were used to indicate the significance of the relationships studied.

THE EXPERIMENTAL TREES

Greater reliance may be placed on the relationship of any growth measurement to yield if it can be shown that the relationship persists with trees under different cultural, climatic, or other conditions. However, the labor and expense involved as well as the necessity of having the trees under control automatically restricted the study to two groups of trees. Under these conditions it seemed desirable to eliminate possible differences in soil, rainfall, elevation, etc., and to consider only the simpler contrasts of age and general vigor. The Fukuda trees had been under observation for some time and, consequently, the choice of a second group (the Akamatsu trees) was determined by the location of the first. The two plots are located near Kainaliu, North Kona. The soil is of approximately the same type, depth, and general physical and chemical characteristics at both locations. The distance between them is 1,000 to 1,500 yards and the difference in elevation only 75 to 100 feet, so that rainfall and temperature factors would be almost identical. The average elevation of the two is approximately 1,500 feet, the annual rainfall about 60 inches.

THE FUKUDA TREES

The Fukuda trees make up a fertilizer experiment, established in 1930, consisting of a 5 by 5 Latin square having nine trees per plot.

The trees were 12 years of age and had been under treatment 7 years when the present study was made. Fertilizer had been applied regularly at a basic rate of 160 pounds of N, P₂O₅, and K₂O per acre. A certain amount of renewal pruning, consisting of removing an old vertical and training a new one to take its place, had been practiced; consequently each tree contained one or more young verticals varying in age from 1 to 5 or more years. The trees had received little or no corrective pruning such as thinning out or heading back of the fruiting wood.

Potash proved to be a limiting factor in this experiment, as there are marked differences in growth and yield between those trees that received potash and those that did not (table 1). It may be seen that the potash-fertilized trees yielded approximately eight times as much fruit as the trees receiving no potash, the ratio being the same in both years in spite of the fact that the yield in 1937 was approximately three times greater than in the previous year.

TABLE 1.—*Size, age, and yield of coffee trees grown under different fertilizer treatments—Fukuda experiment*

Treatment ¹	1936 yield per tree	1937 yield per tree	Average number of verticals per tree	Sum of total areas of cross section of verticals per tree	Average area of cross section per vertical	Sum of total ages of verticals per tree	Average age per vertical
	<i>Pounds</i>	<i>Pounds</i>	<i>Number</i>	<i>Square inches</i>	<i>Square inches</i>	<i>Years</i>	<i>Years</i>
NPK-----	17.0	58.0	2.8	9.33	3.3	18.5	6.6
NK-----	16.0	51.0	2.8	8.67	3.1	20.7	7.4
NP-----	2.7	8.5	2.5	4.36	1.7	14.4	5.8
N-----	2.7	5.0	2.5	3.83	1.5	13.2	5.3
No fertilizer (check)-----	3.7	8.8	2.4	4.07	1.7	13.7	5.7

¹ Fertilizers were applied at the rate of 160 pounds of each element per acre, one-half being applied in January and one-half in July of each year.

Visual differences in tree behavior between the potash and no-potash treatments are even more striking than may appear from the table. The no-potash plots invariably appear in poor foliage and the leaves are yellow and mottled even though the trees may be high in nitrogen. The lateral branches make very poor growth, and many die back to the vertical. Elongation as well as radial growth of the vertical after the first 2 years is slow, with the result that a 10- to 12-year-old vertical may be no longer and no greater in diameter than a 3-year-old vertical of a thrifty tree. Also, the verticals are weak structurally, as indicated by a tendency to bend over, even with very light crops.

In contrast, the potash-treated trees are extremely vigorous; their foliage is dark green and waxy, with little or no evidence of mottling, and persists, even on the 2-year-old lateral growth, until the fruit is ripe. Primary and secondary lateral growth is abundant and strong with little or no evidence of dieback. New verticals make strong heavy growth, especially when developing in full sunlight.

It may be seen from table 1 that there are no significant differences as yet apparent between the two treatments containing potash (NPK and NK). In later discussions of or reference to the Fukuda

trees, the potash-treated trees alone are considered. The no-potash trees were so poor as to be unsuited to the experiment planned.

THE AKAMATSU TREES

The Akamatsu coffeetrees were selected in 1937. They were carefully surveyed and the "off-type," replant, diseased, or other non-typical trees were discarded in order to reduce as much of the variability between the two groups as possible. Of these trees, 456 were amenable to relatively accurate measurement. The trees had been planted in 1930 and trained to a multiple-vertical system. Little or no renewal pruning had been practiced, and, in consequence, all the verticals were approximately of the same age. The trees had received no other pruning except that the lower lateral branches had been pruned off for a distance of about 4 feet above the ground. Practically no fertilizer had been applied during the 2 preceding years so that the trees were in relatively poor foliation and a low state of vigor and appeared to be deficient in nitrogen. Fertilizers were applied in July 1937, but since the rate was low and the trees were in full crop for the year it was thought that this fertilization would not affect the lateral and terminal growth of that season.

COMPARISON OF GROWTH AND YIELD MEASUREMENTS OF THE FUKUDA AND AKAMATSU TREES

The Fukuda and Akamatsu trees exhibit striking differences in growth and yield characteristics, as may be seen from table 2, in which the means of the growth and yield variates, with standard errors, are given. It will be noted that in 1937, which was a year of high yields, the Akamatsu trees yielded less than half as much as the highly fertilized Fukuda trees. The 1937 lateral growth was shorter on the Akamatsu trees in spite of the fact that they were younger and carried a much lighter crop. The 1936 terminal growth was considerably shorter which, although the yield for that year is unknown, is significant in view of the fact that the age of the trees was only 7 years as compared with the 12 years of the Fukuda trees. It will be indicated repeatedly, however, that the estimate of terminal growth of the Fukuda trees was influenced by the pruning practice.

TABLE 2.—Means with standard errors of the variates used in the analyses of the growth and yield of the Akamatsu and Fukuda coffeetrees

Variate	Akamatsu, mean	Fukuda, mean
1937 yield per tree.....pounds..	22. 645±0. 613	54. 425±1. 906
1936 yield per tree.....do.....		17. 015±1. 144
1937 average lateral growth.....inches..	10. 170±. 111	12. 322±. 240
1937 average terminal growth.....do.....		12. 056±. 498
1936 average terminal growth.....do.....	13. 050±. 634	22. 430±1. 062
1937 total area of cross section of verticals per tree.....square inches..	9. 803±. 431	9. 024±. 496

The area of cross section of verticals, although the difference is not statistically significant, was somewhat greater for the Akamatsu trees than for those at Fukuda. This difference is doubtless due to the fact that no renewal pruning had as yet been practiced on the Akamatsu trees and all or most of the fruiting verticals were 6 to 7 years of age, while the Fukuda trees had one or more young verticals on almost

every tree. The Fukuda trees, therefore, have a wider range in age of verticals, which is also expressed in the greater variability of the cross-sectional areas of the verticals per tree in proportion to the mean.

INTERRELATIONSHIPS OF GROWTH AND YIELD OF THE AKAMATSU TREES

The interrelationships of individual tree yield and growth responses of the Akamatsu trees were studied by calculating partial and simple regression coefficients and their sampling errors, the individual tree being used as a unit. The best simple expression of the independent relationships of yield on the several estimates of tree growth and size is provided by the partial regression coefficients (line 1, table 3) according to the equation

$$(y-\bar{y})=b_l(l-\bar{l})+b_t(t-\bar{t})+b_c(c-\bar{c})$$

where y =1937 individual-tree yield of cherry coffee in pounds;

l =1937 average lateral growth per tree in inches;

t =1936 average terminal growth of verticals per tree in inches;

and c =sum of the squared circumferences of verticals per tree in 1937 in square centimeters.

A number of the simple regression coefficients were calculated also and are given in table 3, lines 2 to 7, with sampling errors. A study of these coefficients, in relation to their sampling errors, shows that, with the exception of the regression coefficient of 1936 average terminal growth on the average sum of squared circumferences, all the coefficients are highly significant. The partial regression coefficients (line 1) indicate the independent relationships of the variates to the 1937 yield. For example, when the squared circumferences and 1936 terminal growth are held at their averages there is a negative relation between the yield (i. e., the developing crop) and the lateral growth produced in the same season. Likewise, when the squared circumferences and 1937 lateral growth are held at their means it is found that the 1937 yield is positively related to the previous season's (1936) terminal growth. Thus it is to be expected that the 1937 lateral growth would bear a negative relation to the 1936 terminal growth (line 5).

TABLE 3.—Simple and partial regression coefficients of coffeetree measurements in various relationships, together with their standard errors—Akamatsu experiment

Dependent variate	Regression coefficients (b) of dependent variate on indicated independent variates			Standard errors of regression coefficients		
	1937 average lateral growth	1936 average terminal growth	Average sum of squared circumferences per tree	1937 average lateral growth	1936 average terminal growth	Average sum of squared circumferences per tree
	<i>Inches</i>	<i>Inches</i>	<i>Square centimeters</i>	<i>Inches</i>	<i>Inches</i>	<i>Square centimeters</i>
1937 yield.....	1-1.933	1+0.605	1+0.027 1+.028	0.205	0.111	0.002 0.002
	1-2.312	1+.801		.351	.092	
1937 average lateral growth.....			1-.0055			.0018
		1-.292			.102	
1936 average terminal growth.....			+.001			.001

¹ P value<0.01.

When other variates are held at their means, yield is positively related to size of tree as measured by the sum of the squared circumferences of verticals (line 1). However, it might be expected that annual increments of increase in area would be of more interest when these can be obtained. The simple regressions are of little interest considering the strong independent relationships indicated by the partial regression coefficients. It remains to be determined whether the 1937 lateral growth will bear a positive relationship to the 1938 yield.

INTERRELATIONSHIPS OF GROWTH AND YIELD OF THE FUKUDA TREES

The interrelationships of growth and yield of the Fukuda trees were studied also. The independent effects of the five growth and yield variates employed are provided by the partial regression coefficients in the equation

$$(y - \bar{y}) = b_{y_1} (y_1 - \bar{y}_1) + b_l (l - \bar{l}) + b_t (t - \bar{t}) + b_{t_1} (t_1 - \bar{t}_1) + b_c (c - \bar{c})$$

where y = 1937 individual-tree yield of cherry coffee in pounds;
 y_1 = 1936 individual-tree yield of cherry coffee in pounds;
 l = 1937 average lateral growth per tree in inches;
 t = 1937 average terminal growth of verticals per tree in inches;
 t_1 = 1936 average terminal growth of verticals per tree in inches;
 and c = sum of square circumferences of all fruiting verticals per tree in 1937 in square centimeters.

These coefficients are given in line 1 and a number of the simple regression coefficients that were calculated are given in lines 2 to 15 of table 4.

TABLE 4.—Simple and partial regression coefficients of coffeetree measurements in various relationships together with their standard errors—Fukuda experiment

Dependent variate	Regression coefficients (b) of dependent variate on indicated independent variates					Standard errors of regression coefficients				
	1936 average yield per tree	1937 average lateral growth	1937 average terminal growth	1936 average terminal growth	Sum of squared circumferences per tree	1936 average yield per tree	1937 average lateral growth	1937 average terminal growth	1936 average terminal growth	Sum of squared circumferences per tree
	Pounds	Inches	Inches	Inches	Square centimeters	Pounds	Inches	Inches	Inches	Square centimeters
1937 yield	-0.158 1 - .627	-2.822 1 - 3.922	+1.378 1 - 1.500	+0.322	+0.782	0.174 .167	0.759 .742	0.473 .119	0.203 .192	0.367 .411
1936 yield				+0.069	- .001				.116	.248
1937 lateral growth	+ .574			+1.064	+ .126	.132			.315	.335
1937 terminal growth	+ .168			+ .247	+ .147	.043			.057	.107
1936 terminal growth					- .426					.226

¹ P value < .01.

Keeping in mind the age and vigor of these trees, an examination of the partial regression coefficients of 1937 yield (y) on each of the independent variates in turn when the other variates are held at their means, brings out a number of pertinent facts. The partial regression coefficient of the 2 years' yields shows that the 1937 yield is not proportionately related to the 1936 yield. Apparently the size of the 1936 crop sets up other tree reactions which may be either positively or negatively associated with the succeeding year's yield, and when these are held constant the yield per tree will vary more or less at random. This is borne out by the fact (line 2) that the 1937 yield, when considered in relation to the 1936 yield alone and without regard to other tree responses, is in significant negative relationship. From this relationship, the further assumption may be made that, no matter how great the yield of a tree, if other growth reactions can be maintained or increased, the yield in the succeeding year may also be maintained or increased, weather factors permitting.

Following this argument, the 1937 yield should be significantly and positively proportional to the 1936 terminal growth (lines 1 and 5) and the 1936 yield and 1936 terminal growth (line 6) should also be proportional. In contrast to significant coefficients obtained with the Akamatsu trees, these are not statistically significant. It is believed that with these older trees, particularly when verticals of widely different ages may be included in each tree, the vertical growth measurements made are not truly representative of the cropping capacity of the tree.

The strong negative regression coefficients of 1937 lateral and terminal growth on 1937 yield (lines 1, 3, and 4) indicate that in this year the generally heavy crop per tree had a marked influence on the length of growth in the same year and by inference on the potential crop of 1938.

When other variates are held at their means the 1937 yield per tree is related to size of tree as measured by sum of squared circumferences of fruiting verticals (line 1). However, with trees of this age and growth status it appears that seasonal variations in yield may not be estimated from size of tree as none of the simple regression coefficients (lines 6, 8, 11, 14, and 15) is statistically significant. This lack of correlation was mentioned in discussing the similar relationships with the Akamatsu trees, where, however, the simple regression coefficients were found to be statistically significant (lines 2 and 6, table 3).

DISCUSSION

The extreme differences in mean yields of cherry coffee of the Fukuda trees in the 2 years (1937, 54 pounds; 1936, 17 pounds) and between the Fukuda and Akamatsu trees in the same year are of considerable importance from the standpoint of a growth and yield study. While the data presented do not offer a full explanation or interpretation, as several important relationships were not obtained, the methods employed and the relationships found appear to have promise of value in interpretation of tree responses.

The 1936 terminal growth was the only growth measurement made that may be correlated directly with both 1936 and 1937 yields. Relationships of terminal growth and yield appear to have certain limitations, for with the Akamatsu trees, which are young and rela-

tively low in vigor, the 1936 terminal growth is significantly related to the 1937 yield, while with the Fukuda trees the 1936 terminal growth is related neither to the 1936 nor to the 1937 yields although the 1937 terminal growth is significantly related to the 1937 yield. It appears that the terminal growth of trees of the age and condition of the Fukuda trees is not a sensitive index of the actual yielding capacity except in a year of producing a heavy crop. During 1936, which was a light crop year and favorable for growth, the trees did not respond differentially in terminal growth as they did in 1937 with a much heavier crop. The terminal growth of a 12-year-old vertical which, under normal conditions is approaching maximum height, is relatively short even though the main body of the tree is making vigorous growth. Also, a number of these trees had been subjected to some renewal pruning (consisting of removing an old vertical, thus permitting a new vertical to grow and replace it), and by including in the average the growth of the younger verticals, the differences in growth of the older verticals and relationships with yield were obliterated. Thus terminal growth is an accurate index of the potential crop of the following year when younger, unpruned trees are used, but it does not apply to older trees which have undergone a certain amount of renewal pruning.

The strong negative regressions of 1937 lateral growth and 1937 yield of both the Akamatsu and Fukuda trees corroborate the negative terminal growth relationships found and are indicative of what may have occurred, in part at least, in 1936 to account for the difference in yield in the 2 years. All the fruit is borne on lateral wood, and in trees 12 years of age it is obvious, by inspection, that the greater bearing area is in the lower two-thirds to three-fourths of the typical tree. Therefore, the lateral growth in this area should, perhaps, be a better index of potential crop than the terminal growth even though the two are closely related, as may be seen.

Dean (4) has shown that the spring rainfall of the preceding growing season is a dominant factor in determining coffee yields in the Kona district. This can be explained by assuming that seasonal effects may be carried over from one season into the next through some growth and storage response of the plant. De Haan (6) indicates that flowering of the coffeetree is dependent upon spring rains. In the Kona district spring rains are most uncertain and irregular, as Dean (4) has pointed out. Thus, if light spring rains do not favor abundant flowering and fruit setting, the tree, carrying only a light crop, would respond to the fertilization and normal summer rains by producing abundant fruiting wood for the following season's crop. This phenomenon may have occurred in 1936 to account for the small yield of that year and the succeeding large crop in 1937. However, even with favorable conditions in 1938 it would seem logical to expect that the 1938 crop of the higher yielding trees of 1937 will be smaller because of the relatively poor growth made in 1937. This is supported by the strong negative regression of 1937 yield on 1936 yield per tree even though the 1936 yields on the average were far below those of 1937, and by the negative relationship of 1937 yield on 1937 lateral growth.

There is abundant evidence also that individual-tree condition and cropping ability are expressed, to a greater or less degree, in spite of favorable or unfavorable climatic factors which are usually general

in nature and affect an entire district. The striking regularity of "on" and "off" years, indicated by the data presented by Dean (4) and McClelland (9), suggests that an occasional extreme seasonal factor may set up a biennial-bearing tendency or rhythm similar to that of apples, reported by Auchter and Schrader (2), Potter (11), and others.

When all other independent variates are held at their means, the size of both young and old trees, as measured by squared circumferences of verticals, is clearly related to yield. The total area of cross section of verticals, however, does not appear to be as logical a basis for prediction of annual yields as, for instance, the annual increment of increase. Such a measurement may be expected to bear the same relationship to yield as lateral growth, or as terminal growth of young trees. This measurement may easily be obtained if proper precautions are taken. However, it cannot be used as readily in the field as the lateral or terminal growth. Annual increments of increase in area of cross section of other fruit trees have been found useful by many investigators.

The characteristics of the lateral growth have not been studied in detail, but observation indicates that the diameter and internode length of the lateral, the color and size of leaf at the node, and perhaps other factors may be related to the fruitfulness of the lateral. Thus the length is not necessarily the most important characteristic as, indeed, is obvious from the long willowy growths with long internodes and thin, light-green leaves that develop in the dense shade of the interior of the tree and seldom produce more than one or two berries at a node. A similar type of growth is found in closely planted orchards, particularly on the lower parts of the tree. These observations further emphasize the need of detailed examination of the tree and its response under different conditions in order that constructive and economically sound cultural practices may be developed.

CONCLUSIONS

This study of the yield and growth measurements of two groups of coffeetrees—the Akamatsu trees, which are 7 years of age and relatively low in vigor and production, and the Fukuda trees, which are 12 years of age and high in vigor and production—shows that, with minor exceptions, the same relationships exist in both groups. Thus it may be concluded:

- (1) That certain growth responses of the tree are largely dependent upon or conditioned by the size or volume of the developing crop.

- (2) That the volume of the crop is largely determined by the growth made in the preceding growing and crop season.

- (3) That a dominant weather factor, such as spring rains, may disturb these relationships, as Dean (4) has shown, but the tree will resume its normal, overlapping, 2-year growth-and-bearing cycle in succeeding average years.

- (4) That by judicious pruning and fertilization—the first of which would tend to reduce the current or immediate year's crop and both of which would tend to increase the production of vigorous fruiting wood—and perhaps by other cultural practices such as mulching which would tend to conserve moisture, the extreme fluctuations in annual yields may be reduced and the average yield as well as the general size and vigor of the tree may be considerably increased.

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SEASONAL ABUNDANCE OF THE CORN EARWORM¹

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INTRODUCTION

Estimates of the abundance of the corn earworm (*Heliothis armigera* (Hbn.)³), also called the tomato fruitworm and the cotton bollworm, are commonly based on the degree of infestation observed on the affected hosts or, in some instances, on the number of complaints of injury received from growers. Workers engaged in the study of this pest have usually gaged its abundance during its active season by making egg counts at regular intervals, by measuring the larval injury throughout the season, or by a combination of these two methods.

In central Virginia and the territory adjacent to the District of Columbia, the areas included in this study, the great mass of the corn earworm population occurs on corn (*Zea mays* L.). It is generally agreed that corn is the preferred host plant, and also that the fresh silk is the most attractive part of the corn plant for oviposition. Except in the late-maturing fields, where egg laying is prolonged because more attractive host material is not becoming available, in a particular cornfield oviposition reaches its maximum at or shortly after the time when the corn reaches its maximum silking stage. The moths emerging from hibernating pupae early in the season, before corn becomes attractive for oviposition, deposit eggs on a variety of hosts, a common one being tomato. With the appearance of early attractive corn there is a shift to this plant, and the insect remains concentrated on corn for about 2 months, or until the grain in late-maturing fields hardens, when there is again a decided shift to a variety of hosts, common examples of which are tomato, alfalfa, and particularly cotton in the South.

In 1932 work was begun in the vicinity of Charlottesville, Va., to develop methods for obtaining a better understanding of the seasonal population on corn. With the discontinuance of the Charlottesville laboratory in 1933, this study was suspended until 1934, when observations were resumed at Arlington, Va., adjacent to the District of Columbia. In this area, in addition to seasonal abundance, the factors that affect the abundance from year to year were also given consideration.

The results of these investigations are of particular value in the

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² The writer is indebted to W. J. Phillips, in charge of the Charlottesville, Va., laboratory and to A. H. Madden for aiding in the field work in 1932; to W. H. Larrimer, in charge of the laboratory at Arlington, Va., in 1934 and 1935, for assistance in connection with the project; and to F. W. Poos for helpful suggestions in connection with the field work and preparation of the manuscript.

³ This is now considered to be the correct scientific name for the corn earworm instead of *H. obsoleta* (F.). See the following: HEINRICH, CARL, THE PROPER NAME FOR THE CORN EARWORM. Jour. Econ. Ent. 32: (In press.)

interpretation of the infestations in varietal test plots, where the evidence of relative susceptibility to oviposition may be distorted by a difference in maturity of the corn.

REVIEW OF LITERATURE

The published accounts of the seasonal life history of *Heliothis armigera* reveal a difference of opinion as to both the number of generations⁴ per year and the time of the appearance of the various generations. The usual procedure for determining the life span of a generation has been by interpolation of data on egg counts made at regular intervals and seasonal rearings made in the insectary. It is generally agreed that under optimum conditions during the summer a generation is completed in about a month.

Quaintance and Bishopp (13)⁵ estimated the number of generations occurring annually in the Cotton Belt as from four to seven, depending on the latitude. Barber (1) observed from one to six generations in southeastern Georgia, with generations overlapping so that they were indistinguishable, and he found that dormancy in pupae began as early as June. Isely (8) estimated the number of generations in Arkansas as probably four. He reviewed the records on the depredations of *Heliothis armigera* as a pest of corn, cotton, soybean, grain sorghums, tomato, and alfalfa over the 25-year period 1911-35, and stated that severe damage occurred in the State on one host or another in 9 of these years. Of interest is his observation that the outbreaks were most frequent in a group of counties in which the acreages of corn and of cotton were about equal and that a county bordering this group, in which the acreage of cotton was about four times that of corn, was never involved in an outbreak.

Northward from the Cotton Belt *Heliothis armigera* normally decreases in abundance and is most common on corn, tomato, and alfalfa. Garman and Jewett (6) estimated three generations at Lexington, Ky., between June 10 and September 12, and a fourth imperfect generation early in November. In a study of the seasonal fate of eggs of the corn earworm at Charlottesville and Richmond, Va., during the 4-year period 1924-27, Phillips and Barber (10) found that the percentage of eggs hatching between July 1 and September 1 did not vary greatly.

Headlee (7) gave an account of the seasonal abundance of eggs in the field at Manhattan, Kans., in connection with a study for determining the best planting date for field corn to avoid earworm injury. From rearings and regular egg counts he estimated that there were three generations and a partial fourth which did not mature. He considered that the great increase in the number of eggs per plant during the latter part of August and September was due to the great abundance of the third brood of moths. The corn planted on May 1 was found to bear the least injury. Similar studies continued by McCulloch (9) at Manhattan, Kans., from 1913 to 1918 led to substantially the same conclusions. He fixed the time of maximum emergence of the broods as follows: First, June 15; second, July 13, and third, the last days of August and the first days of September. In

⁴ The term "generation" in this paper is used to include a complete life cycle from the egg to the adult. The term "brood" is applied to a stage of the insect within a life cycle; e. g., the moths emerging from the overwintering pupae are the first brood of moths.

⁵ Italic numbers in parentheses refer to Literature Cited, p. 257.

comparison with the third generation, the first and second generations were considered of little importance.

Ditman and Cory (3, 4) concluded that in Maryland there are usually not more than two generations, although theoretically moths emerging early in the spring would produce three generations, or even four, in a long summer season. They further concluded that "generations overlap to such an extent that there is a regular and gradual building up of the corn earworm population from the beginning to the end of the season." Ditman and Cory (5) observed a slight depression in infestation in sweet corn maturing the last week of July and the first week of August from that in earlier maturing corn.

In central Virginia Phillips and Barber (10) found that in corn planted at weekly intervals from April 18 to June 26 egg deposition was usually the lightest in the plantings from May 8 to 29. Plantings from April 18 to May 2 usually received the next largest number of eggs, and the plantings in June the largest number. These writers concluded that corn planted in midseason (May 8-29) received the fewest eggs because it silked between the appearance of the first and second broods of moths. They consider the second brood as the one causing the heavy infestations in the late-planted corn coming into silk the latter part of August. In the same area Phillips and Barber (11) found the smallest reduction in yield due to the corn earworm, both in experimental plots and in the field, in corn planted before May 15.

EXPERIMENTS AT CHARLOTTESVILLE, VA.

EGGS

In earlier experiments at Charlottesville Phillips and Barber (11) found that infestations in contiguous plantings of corn made at regular intervals were heavier than those occurring under field conditions. They believed this to be brought about by a concentration of ovipositing activity in the small early plantings, resulting in the building up of an infestation in the later plantings by successive generations. To avoid this unnatural condition in the experiments conducted during 1932, plots of sweet corn (*Zea mays* var. *rugosa* Bonafous), planted at more or less regular intervals were established in cornfields in the vicinity of Charlottesville, separated far enough from one another to eliminate the chance of building up an infestation adjacent to each planting. Egg counts were made at random in these plantings on silks attractive for oviposition (2 to 5 days after they emerged from the ear shoot) and on some additional smaller plantings that silked after this group had ceased to be attractive for oviposition. Egg counts were made every other day, the number of samples depending on the number of plots in fresh silk. The size of the samples ranged from 5 to 30 silks, most of the larger samples being taken in midsummer, when the rate of oviposition per silk was low. Table 1 summarizes these data for the period July 12 to October 29, inclusive.

The number of eggs deposited per silk decreased abruptly following the 10-day period July 12-21 and did not increase appreciably until the latter part of August. The rate of oviposition continued at a high level throughout September, reaching a peak during the period September 20-29, after which there was an abrupt decrease, the last egg being recorded in the period October 10-19. The seeming decrease

in the abundance of eggs the latter part of July and the early part of August at first appeared to be due to a scarcity of moths. General observations in the surrounding area, however, indicated that the increased abundance of field corn attractive for oviposition resulted in a decrease only in the number of eggs per silk.

TABLE 1.—*Seasonal abundance of eggs of the corn earworm on separated plantings of sweet corn in cornfields near Charlottesville, Va., 1932*

Period of sampling	Sam- ples	Silks	Eggs	Average eggs per silk	Period of sampling	Sam- ples	Silks	Eggs	Average eggs per silk
	Num- ber	Num- ber	Num- ber	Number		Num- ber	Num- ber	Num- ber	Number
July 12-21.....	11	75	75	1.00	Sept. 10-19.....	6	35	77	2.20
July 22-31.....	19	95	17	.18	Sept. 20-29.....	5	25	60	2.40
Aug. 1-10.....	20	150	16	.11	Sept. 30-Oct. 9.....	5	25	13	.52
Aug. 11-20.....	30	275	28	.10	Oct. 10-19.....	5	25	1	.04
Aug. 21-30.....	17	110	33	.30	Oct. 20-29.....	4	20	0	.00
Aug. 31-Sept. 9.....	13	76	170	2.24					

LARVAL INFESTATIONS IN SEASONAL PLANTINGS

The percentage of ears infested with larvae of the corn earworm in the separated field plots on which egg counts were made on the silks as recorded in table 1 is shown in table 2. The trend of the infestation was from high in plots reaching maximum silking July 15-25 to low in plots reaching maximum silking August 5-9, followed by a rapid increase in the plots reaching their maximum silking after that date.

TABLE 2.—*Percentage of earworm-infested ears in seasonal plantings of sweet corn made in separated cornfields near Charlottesville, Va., 1932*

Planting date	Period when fresh silks were present	Date of maxi- mum fresh silks	Ears ex- amined	Ears in- fested
			Number	Percent
Apr. 21.....	July 8-22.....	July 15	54	68.6
May 3.....	July 19-30.....	July 25	100	93.0
May 17.....	July 18-Aug. 3.....	do	54	75.9
May 30.....	July 24-Aug. 17.....	Aug. 5	100	23.0
June 9.....	Aug. 1-19.....	Aug. 9	100	19.0
June 20.....	Aug. 9-Sept. 2.....	Aug. 21	100	35.0
July 2.....	Aug. 18-Sept. 4.....	Aug. 26	100	72.0
July 15.....	Aug. 30-Sept. 11.....	Sept. 5	55	94.5

The seasonal percentage of ear infestation and the seasonal abundance of eggs are shown graphically in figure 1. It will be readily seen that there is a direct correlation between these two factors. General observations in the vicinity of Charlottesville showed that the period July 22-August 20, when there was a seeming scarcity of eggs and a resultant low percentage of ear infestation, was the period when most of the acreage of field corn was in the stage of silking most attractive to the moths for oviposition. Over the area as a whole the earworm was much more abundant during the period of low percentage infestation than was shown by either the number of eggs per silk or the percentage of ear infestation. Moreover, there was evidence that the

high infestations found in plots early in July, late in August, and in September were not an accurate measure of the general abundance of the earworm or the aggregate injury to corn during those periods. It was obvious that a record of the seasonal abundance of corn attractive for oviposition was essential to the determination of the seasonal abundance of the insect as well as of its degree of injury.

EXPERIMENTS AT ARLINGTON, VA.

OBSERVATIONS ON LIFE HISTORY

In northern Virginia the corn earworm hibernates more or less successfully. During the period 1933-37 pupae survived the winter in hibernation cages at Arlington Experiment Farm each year. In this period there were two winters with below-normal temperatures (one of which was severe), one winter with temperatures slightly above normal, and one very mild. The earliest emergence of moths

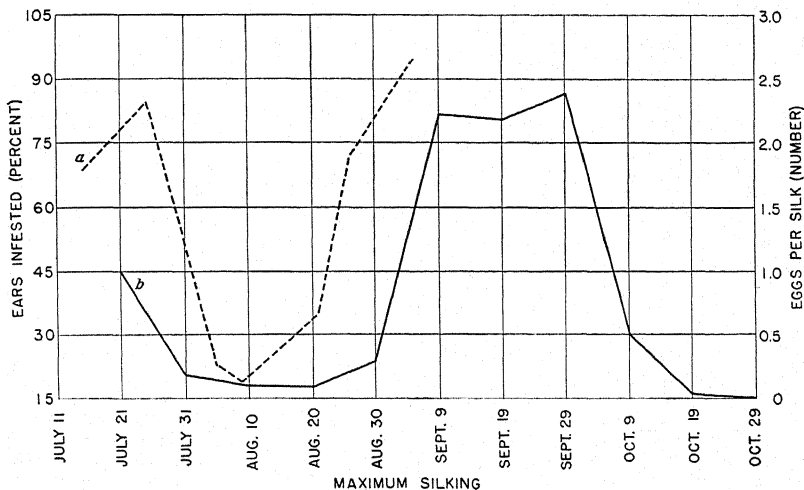


FIGURE 1.—Seasonal percentage of ear infestation by the corn earworm in relation to the seasonal abundance of eggs per silk, Charlottesville, Va., 1932: *a*, Percentage of ears infested; *b*, number of eggs per silk.

in hibernation cages was on June 4, in 1937, and the latest emergence on July 14, in 1934. Although pupae survived the severe winter of 1935-36, no emergence of moths was recorded that year. At Charlottesville, Va., where the climate is somewhat milder than at Arlington, the earliest emergence of moths recorded by Barber and Dicke (2) during the period 1928-33 was on May 25, 1933, and the latest emergence on August 5, 1929. More than 50 percent of the moths emerging from hibernation in cages issued after July 1. Field observations at Arlington during the past 4 years indicate that the effect of cage conditions is to delay emergence of moths from overwintering pupae.

Observations on the first appearance of eggs in the spring were made at Arlington during the period 1934-37. In 1934 the first eggs were recorded on May 18, in 1935 on June 3, in 1936 on May 22, and in 1937 on May 28. In 1935, 1936, and 1937 corn started in the greenhouse in March was used to attract the earliest appearing

moths. In 1937 corn with attractive silks was available in field plots after May 10, but no eggs were found until May 28.

Many workers have made rearings of the corn earworm and have found that a generation is completed in approximately 30 days when eggs are deposited late in June, in July, and early in August. A generation developing from eggs deposited by early-emerging moths late in May requires longer, as do the offspring of moths emerging after August 15. The time required to complete a generation on green corn during the summer varies to a large extent with the temperature. In 1934 insectary rearings were made for two complete and a partial third generation. Eggs deposited June 15, which was almost a month after the first eggs were found in the field that year, produced mature larvae which entered the soil by July 1, and issued as moths from July 15 to 21. Larvae obtained from eggs deposited by these moths on July 17 and 18 became full grown and entered the soil from July 28 to August 10, and the moths issued from August 14 to September 10. Larvae from eggs deposited the third week of August became full grown during the fall of 1934. There was no emergence of moths from individuals placed in cages during the second week of September.

Under field conditions in 1936, on Golden Cross Bantam sweet corn with egg deposition as early as May 22, the first larvae entered the soil for pupation on June 16. In 1937, on similar sweet corn with egg deposition beginning May 28, the first larvae entered the soil on June 14.

Without doubt the second brood of moths was abroad in the field in northern Virginia the first week of July during 1936 and 1937, and the first progeny of this generation, or the third brood of moths, appeared early in August. These observations confirm the findings of McColloch (9) in Kansas and of Ditman and Cory (3) in Maryland. The overlapping of the first and second generations early in July makes the generations lose their identity and results in a gradual increase in the population with the peak late in the summer. With emergence of moths from hibernation covering about a month and a half, it is doubtful whether peaks in oviposition during July and August definitely signify the maximum moth population of a particular brood. The number of complete generations can consequently be only an estimate. It is believed that in the vicinity of the District of Columbia two generations are usually completed, with a considerable part of a third generation often produced.

LARVAL INFESTATION IN SEASONAL PLANTINGS OF SWEET CORN

With the establishment of the corn earworm project at Arlington Experiment Farm, in 1934 and 1935 plantings were made at intervals of about 15 days, beginning May 1, in a single block. The larval infestations observed in these plots are summarized in table 3. The percentage of infestation showed the same seasonal trend as that at Charlottesville in 1932, the plots planted early and those planted late being most heavily infested.

Upon comparison with the seasonal abundance in field corn for the same years (table 5), the seasonal plantings show a much heavier infestation. As has been pointed out, this apparently is due to a concentration of eggs on a small planting of early attractive corn and a subsequent building up of an infestation in successive plantings.

Successive plantings of small plots consequently do not give a true picture of the seasonal abundance of the earworm.

TABLE 3.—Percentage of infestation of the corn earworm in seasonal plantings of Golden Cross Bantam sweet corn, Arlington Experiment Farm, Va., 1934 and 1935

Planting date ¹	Date of beginning of silking	Date harvested	Ears infested	Planting date ¹	Date of beginning of silking	Date harvested	Ears infested
<i>1934</i>				<i>1935</i>			
			<i>Percent</i>				<i>Percent</i>
May 1.....	July 2	July 25	88.0	May 1.....	July 12	July 27	93
May 16.....	July 16	Aug. 3	61.0	May 15.....	July 17	Aug. 8	52
May 29.....	July 23	Aug. 10	81.8	May 29.....	July 24	Aug. 13	62
June 12.....	July 30	Aug. 20	87.8	June 11.....	Aug. 5	Aug. 21	77
June 26.....	Aug. 13	Sept. 4	99.3	June 25.....	Aug. 12	Aug. 29	88
				July 10.....	Aug. 28	Sept. 27	100

¹ In 1934, 4 plots were planted on each date and in 1935 only 1 plot. In each plot 100 ears were examined.

SEASONAL ABUNDANCE OF EGGS IN RELATION TO SEASONAL ABUNDANCE OF CORN ATTRACTIVE FOR OVIPOSITION

In 1936 egg counts on silks attractive for oviposition were made at Arlington throughout the silking period. The seasonal abundance of corn in attractive silk was similar to that in the representative group of fields included in the field survey in Fairfax County. A sample constituted 15 attractive silks, except in a few instances when plots first came into silk and this number was not available.

Table 4 gives a summary of the abundance of eggs in relation to the corn acreage in attractive silk throughout the season. The same 6-day periods as those classifying the fields covered in the survey in Fairfax County (table 6) were used.

TABLE 4.—Seasonal abundance of eggs of the corn earworm in relation to the abundance of corn attractive for oviposition, Arlington and Fairfax Counties, Va., 1936

Period of sampling	Samples	Silks examined	Eggs found	Eggs per silk	Corn acreage in maximum silk	Numerical rating of abundance of eggs ¹	Seasonal abundance of eggs
	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Percent</i>		<i>Percent</i>
July 18-23.....	8	120	67	0.56	4.9	2.7	4.9
July 24-29.....	4	60	30	.65	23.4	15.2	27.4
July 30-Aug. 4.....	8	120	58	.48	25.1	12.0	21.7
Aug. 5-10.....	5	75	26	.35	39.5	13.8	24.9
Aug. 11-16.....	8	79	130	1.65	7.1	11.7	21.1
Aug. 17-22.....	8	120	210	1.75			
Total.....	41	574	530		100.0	55.4	100.0

¹ Calculated by multiplying eggs per silk by acreage in maximum silk.

The general abundance of corn earworm eggs per silk during the season at Arlington shows a trend similar to that observed at Charlottesville (table 1). When the number of eggs per silk is adjusted according to the percentage of the corn acreage attractive for oviposition, it may be seen that there is a great increase in the abundance of eggs during the second 6-day period and a slight decrease during the following periods, which probably has little significance. Of particular interest is the fact that during the period August 5-10, when the counts showed the fewest eggs per silk, the abundance of eggs was

high when adjusted according to the corn acreage in attractive silk. Moths of the second and third broods unquestionably were very abundant, even though the eggs appeared to be scarce. The great decrease in the acreage of corn attractive for oviposition during the period August 11-16 obviously resulted in a concentration of eggs per silk. Oviposition continues at a high rate in the late-maturing fields and for a longer period than on the earlier maturing corn because of the greatly diminished supply of corn in attractive silk and also

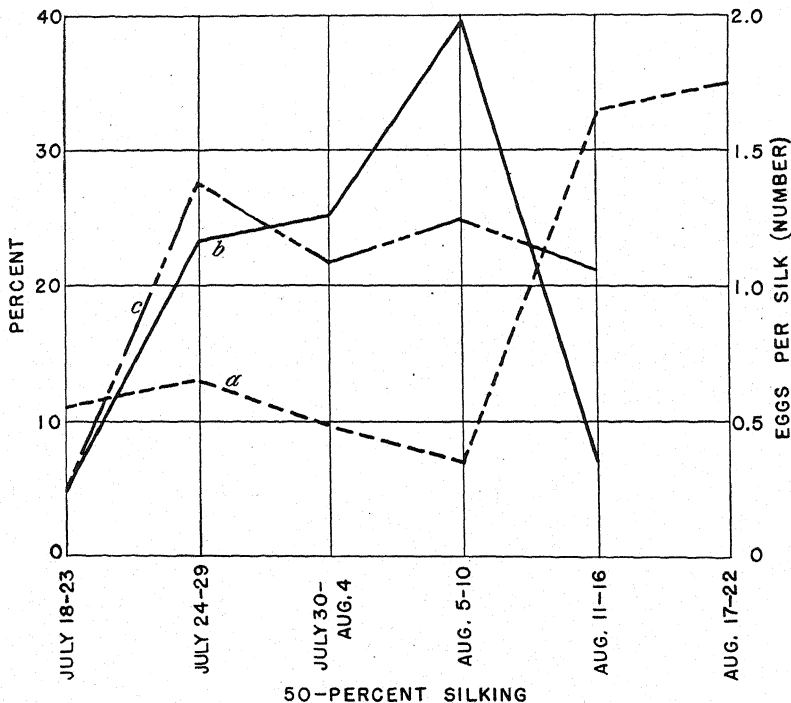


FIGURE 2.—Abundance of eggs in relation to abundance of corn in attractive silk, Arlington and Fairfax Counties, Va., 1936: *a*, Number of eggs per silk; *b*, percentage of acreage in silk; *c*, percentage of total eggs.

because of the retarded rate of maturing due to shorter days and somewhat lower temperatures late in the summer.

The relation between the number of eggs per silk and the abundance of eggs when adjusted according to the percentage of the total acreage of corn attractive for oviposition is shown graphically in figure 2. There is a positive correlation between the seasonal abundance of corn in attractive silk and the seasonal abundance of eggs. It is evident that the peak of the moth population and eggs was reached before it was indicated by the egg counts.

These data, confirming those from Charlottesville, Va. (table 1), show clearly that egg counts in themselves are not an accurate measure of the abundance of the corn earworm on corn, but must be considered in connection with the acreage of corn attractive for oviposition.

SURVEY OF SEASONAL ABUNDANCE IN FIELD CORN, 1934-37

METHODS

Since experience had shown that successive plantings of corn in small plots did not give a satisfactory measure of the seasonal abundance of the corn earworm, methods were developed in which use was made of a group of fields representing the planting period for a community. To obtain a representative sample for the area surveyed, all the fields on both sides of a route established at planting time were chosen for observation throughout the season.

In extensive studies on the egg-laying habits of the earworm moth, Phillips and Barber (10) found that the silks are most attractive for oviposition on the third day after emerging from the ear shoot. Consequently fields normally reach their maximum attractiveness for oviposition at about the time when the maximum amount of fresh silk is present.

In 1934, 1935, and 1936 counts were made to determine the relative maturity of the fields during the silking period. No silk counts were made in 1937, the fields being classified solely on the basis of when they began to silk, which is a more practical method, and probably as accurate as making silk counts. The larval-population counts were made by opening the ears approximately 1 month after the fields came into silk. Ears from which larvae had made their exit to enter the soil for pupation were recorded as infested with one larva. The samples were taken at random. In 1934 and 1935 5 samples of 20 ears each on consecutive culms, in 1937 10 samples of 10 ears each, and in 1936 5 samples of 40 ears each were examined from each field. In 1936 culm counts were also made, so that a better idea could be obtained of the general ear population per acre. In most of the fields the stand was thinned after it was well established, which tended to make the culm population uniform. The culms were counted in 50 feet of row length at the five points where the examinations were made to determine the infestation. With rows spaced at 3.5 feet, as found in all the fields, 250 feet of row length constitutes about one-fiftieth of an acre. Some culms bore two ears, but these were largely offset by barren culms. No multiple-eared varieties were encountered.

One difficulty in determining the seasonal abundance of the corn earworm in a survey of this type is the cannibalistic habit of the larvae, which greatly reduces the larval population in late-maturing fields where oviposition has been concentrated. The infestation counts consequently give a better index of the number of larvae that enter the soil for pupation than of the abundance of moths or eggs.

The larval population for each silking period was found by calculating the acreage in each group of fields that would be 100 percent infested if the infestation were concentrated on such acreage, and then calculating the percentage of the total acreage thus infested for each silking period.

GENERAL CONDITIONS OF THE AREA SURVEYED

In 1934 and 1935 the survey was made in Montgomery County, Md., and in 1936 and 1937 directly across the Potomac River in Fairfax County, Va. This general area has a rather irregular topography and is devoted mostly to general and dairy farming. The corn

earworm population was studied largely in field corn, although a small acreage of sweet corn was encountered. As far as could be determined, the seasonal development of the corn earworm in the two communities surveyed was comparable. The farm practices were similar, although a greater acreage of silage corn in Fairfax County accounted for an increased acreage planted late. All the corn under observation in both areas was cut. In the earliest-maturing fields cutting was begun the latter part of August, and in the late fields cutting was usually completed early in October.

SURVEYS IN MONTGOMERY COUNTY, MD., 1934 AND 1935

The data obtained from the survey in Montgomery County are summarized in table 5. The seasonal abundance of the larvae in relation to the percentage of ears infested and to the corn acreage attractive for oviposition is illustrated in figure 3. There is a slight drop in the percentage of infestation in the fields of the second group in both 1934 and 1935, but since there is a considerable increase in the acreage of corn attractive for oviposition, it is obvious that the earworm population in the area as a whole is increasing rapidly. In both years the maximum larval population occurred in the maximum acreage of corn in attractive silk. In 1935 the percentage of ears infested reached a minimum at the same time that the population of larvae when adjusted to acreage in susceptible silk reached a maximum. With the reduction of the acreage of corn in attractive silk there was also a decrease in larval population, but the percentage of ears infested increased.

TABLE 5.—Seasonal abundance of the corn earworm in Montgomery County, Md., in 1934 and 1935 and in Fairfax County, Va., in 1936 and 1937

MONTGOMERY COUNTY, MD.							
Grouping of fields according to silking period ¹	Fields	Acreage silking		Ears infested	Range of infestation	Acreage with all ears infested	
	Number	Acres	Percent	Percent	Percent	Acres	Percent
<i>1934</i>							
July 9-15.....	2	26	9.0	25.0	24-26	6.5	7.9
July 16-22.....	5	72	24.8	22.0	7-37	15.8	19.1
July 23-29.....	11	163	56.2	29.6	11-52	48.2	58.3
July 30-Aug. 6.....	3	29	10.0	42.0	22-71	12.2	14.8
Total.....	21	290	100.0	29.2	7-71	82.7	28.5
<i>1935</i>							
July 14-20.....	5	68	19.4	24.8	15-37	16.9	16.1
July 21-27.....	15	160	45.7	21.3	6-34	34.1	32.5
July 28-Aug. 3.....	7	64	18.3	39.0	23-60	25.0	23.8
Aug. 4-10.....	5	58	16.6	50.0	39-68	29.0	27.6
Total.....	32	350	100.0	30.3	6-68	105.0	30.0
FAIRFAX COUNTY, VA.							
<i>1936</i>							
July 18-23.....	3	19.0	4.9	25.9	12.0-40.5	4.9	5.3
July 24-29.....	10	90.5	23.4	14.9	5.0-32.5	13.5	14.5
July 30-Aug. 4.....	9	97.0	25.1	17.2	4.5-26.5	16.7	17.9
Aug. 5-10.....	13	153.0	39.5	28.9	15.5-43.0	44.2	47.4
Aug. 11-16.....	5	27.5	7.1	50.8	35.5-64.5	14.0	15.0
Total.....	40	387.0	100.0	24.8	4.5-64.5	93.3	24.1
<i>1937</i>							
July 5-11.....	3	44	12.0	36.7	30-48	16.1	8.1
July 12-18.....	15	118	32.1	31.4	13-53	37.1	18.6
July 19-25.....	16	145	39.4	63.3	33-94	91.8	46.0
July 26-Aug. 1.....	2	16	4.3	62.0	55-69	9.9	5.0
Aug. 2-8.....	2	9	2.4	96.0	93-99	8.6	4.3
Aug. 9-15.....	2	13	3.5	100.0	100	13.0	6.5
Aug. 16-22.....	2	23	6.3	100.0	100	23.0	11.5
Total.....	42	368	100.0	54.8	13-100	199.5	54.2

¹ In 1934, 1935, and 1937 these dates represent the beginning of silking, but in 1936 they represent the 50-percent silking period.

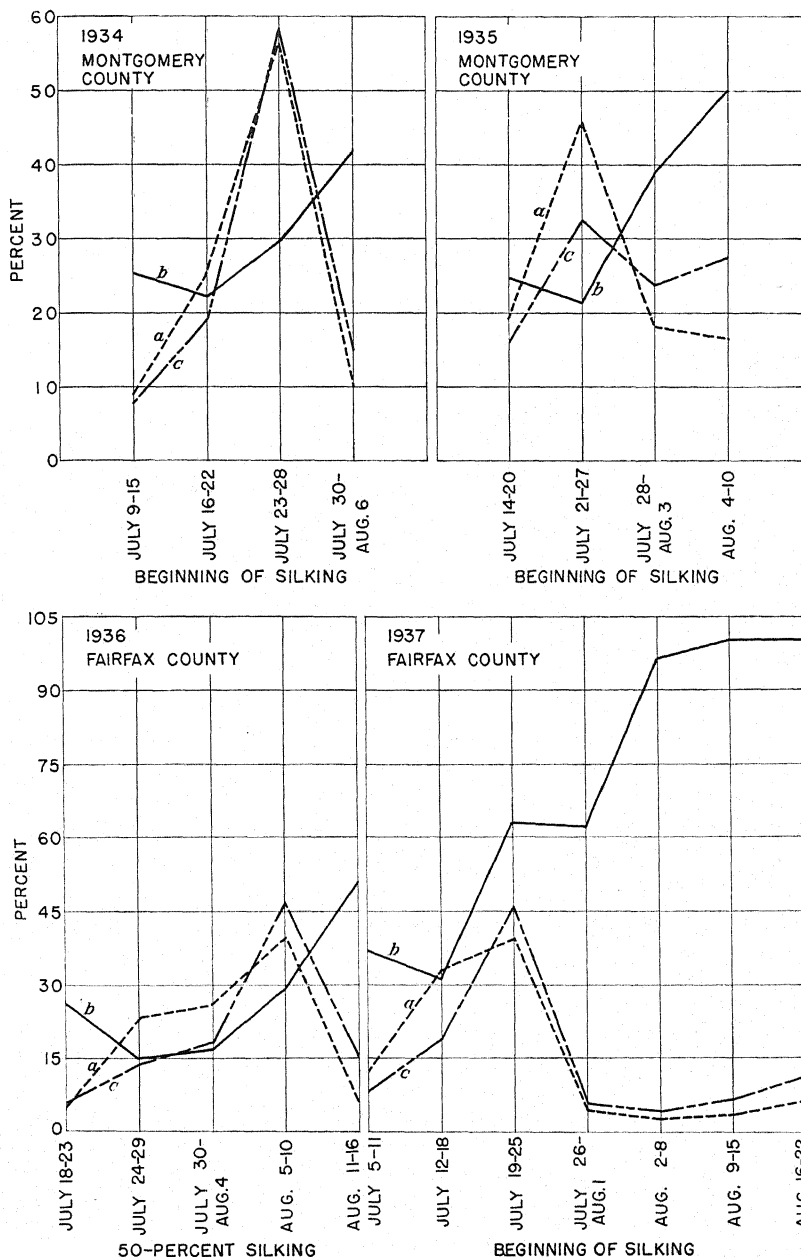


FIGURE 3.—Relation between abundance of the corn earworm and the acreage in silk in cornfields in Montgomery County, Md., in 1934 and 1935, and in Fairfax County, Va., in 1936 and 1937: *a*, Percentage of total acreage silking; *b*, percentage of ears infested; *c*, percentage of total acreage with all ears infested.

SURVEYS IN FAIRFAX COUNTY, VA., 1936 AND 1937

The survey in Fairfax County was along the same route both years, and in most instances on the same farms. The data are summarized in table 5 and figure 3. A detailed summary of the data obtained in 1936 is given in table 6.

TABLE 6.—Detailed summary of the survey of the seasonal abundance of the corn earworm on field corn in Fairfax County, Va., 1936

Grouping of fields according to 50-percent silking period	Size of field	Total culms in field	Ears infested		Larvae per 100 ears	Total larval population			
	Acres	Number	Percent	Number	Number	Number			
July 18-23.....	9 3 7	80,550 21,150 40,250	29.0 40.5 12.0	23,360 8,566 4,830	29.5 41.5 12.0	23,762 8,777 4,830			
Total.....	19	141,950	25.9	36,756	26.3	37,369			
July 24-29.....	6 7 7 3.5 6 8 18 18 9	33,900 46,900 47,600 23,975 37,500 54,000 57,200 134,100 178,200 59,400	9.5 7.5 7.5 8.0 5.0 10.0 9.0 16.5 19.0 32.5	3,221 3,518 3,570 1,918 1,875 5,400 5,148 22,127 33,858 19,305	9.5 7.5 7.5 8.0 5.0 10.0 9.0 16.5 19.0 32.5	3,221 3,518 3,570 1,918 1,875 5,400 5,148 22,127 33,858 19,305			
	Total.....	90.5	672,775	14.9	99,940	14.9	99,940		
	July 30-Aug. 4.....	7 5 25 8 4 9 6 25 8	48,300 41,250 157,500 48,400 29,800 59,400 38,700 158,750 66,400	14.5 8.0 11.5 7.0 20.5 24.0 4.5 26.5 23.5	7,004 3,300 18,113 3,388 6,109 14,256 1,742 42,069 15,604	14.5 8.0 11.5 7.0 20.5 24.0 4.5 26.5 23.5	7,004 3,300 18,113 3,388 6,109 14,256 1,742 42,069 15,604		
		Total.....	97	648,500	17.2	111,585	17.2	111,585	
		Aug. 5-10.....	7 6 7 12 8 12 25 18 7 9 12 20 10	50,750 36,300 53,900 99,600 56,800 122,400 233,750 131,400 53,200 73,350 138,600 120,000 86,000	26.0 20.0 15.5 28.0 27.0 24.5 31.5 26.0 22.0 31.5 27.0 43.0 33.5	13,195 7,260 8,355 27,888 15,336 29,988 73,631 34,164 11,704 23,105 37,422 51,600 28,810	26.0 20.0 15.5 28.5 27.0 24.5 31.5 26.0 23.0 31.5 27.0 43.0 33.5	13,195 7,260 8,355 28,386 15,336 29,988 73,631 34,164 12,236 23,105 37,422 51,600 28,810	
			Total.....	153	1,256,050	28.9	362,458	28.9	363,488
			Aug. 11-16.....	5 4 5 4.5 9	34,500 36,800 30,250 33,975 111,150	35.5 57.0 64.5 37.0 54.0	12,248 20,976 19,511 12,571 60,021	35.5 57.0 74.0 39.0 56.5	12,248 20,976 22,385 13,250 62,800
				Total.....	27.5	246,675	50.8	125,327	53.4
Grand total.....				387	2,965,950	24.8	736,066	25.1	744,041

In general, the seasonal trend of the population, the percentage infestation, and the abundance of corn attractive for oviposition were similar to those of 1934 and 1935 in Montgomery County. In 1936 the decrease in the percentage infestation in the second period was

greater than in any other year. This is attributed to a low moth population, which had not had sufficient time to build up after the heavy mortality of hibernating pupae during the previous winter. Two methods of calculating the seasonal abundance were compared in 1936. There was very little difference in the results of computing the seasonal population on the basis of the acreage 100 percent infested and the larval population based on the percentage of ears infested and the culm population.

After a mild winter the corn earworm was abundant during 1937. The percentage of ears infested was higher in the earliest silking fields than in any of the other years. An extreme example of how a 100-percent infestation in late-maturing fields may obscure the abundance of the insect is illustrated in figure 3. As has been stated, cannibalism among larvae in late-maturing fields makes it difficult to ascertain the abundance of moths or eggs by counts of larvae made approximately a month after a field comes into silk. However, even if the population in late fields were a few times greater than that shown in the graph, the percentage infestation would still be a poor index of the insect's abundance in comparison with the percentage infestation in periods when the acreage of attractive corn was much greater.

DISCUSSION OF SEASONAL ABUNDANCE

The range in the percentage infestation in the various groups of fields for the 4 years is given in table 5. There is, in each group, a rather wide range in percentage of infestation. Each year the trend of the minimum percentage infestation is very similar to the trend of the maximum percentage infestation. Among the factors contributing to this wide range are variations in (1) source of moths, (2) uniformity in varieties of corn, (3) soil fertility and uniformity, (4) temperature and soil moisture, (5) attractiveness of different varieties, and (6) overlapping in maturity of fields between the periods.

That the source of moths is important in this respect is evidenced by the building up of infestations in contiguous seasonal plantings. There is considerable variation in the length of the silking period and the time required to mature in different varieties. Some fields reach their maximum silking period in 8 or 10 days, whereas others require 2 weeks. Soil fertility, temperature, and moisture are important factors in the rate of growth. Different parts of a field irregular in topography vary greatly in maturity at silking time, which results in their being exposed to different moth populations. Variation in attractiveness of different fields is very evident, and was observed between field corn and sweet corn. In grouping the fields according to maturity in weekly periods, overlapping in maturity between periods is unavoidable.

In the area adjacent to the District of Columbia the silking period begins either late in June or early in July, and attractive fresh silks are generally present until the coming of frost, usually in the latter part of October. The first corn to come into silk is a small acreage of early sweet corn on which moths emerging late from overwintering pupae the last of June and early in July, as well as the earliest moths of the second brood, concentrate for oviposition. This results in a high percentage of ear infestation. The earliest field corn, usually only a few small fields in a community, comes into silk about the second

week of July. The infestation in these fields consists primarily of the early-issuing second brood of moths and is normally lower than in fields of early sweet corn, but higher than in fields coming into silk a week later. With the rapid increase in acreage of corn in silk the percentage of ears infested declines but the general population of larvae increases. The rapid increase in the acreage of corn attractive for oviposition has the effect of dispersing the increasing moth population over a large area. As the acreage of corn attractive for oviposition decreases, the population becomes concentrated, resulting in a heavy deposition of eggs on a small late-silking acreage. The population of moths and eggs is normally about as high late in July or early in August, when superficially they appear to be reduced in numbers, as late in August and in September, when they appear to be very abundant.

The minimum infestations found in Kansas by Headlee (7) and McColloch (9) in plantings made May 1 were apparently caused by a rapidly increasing acreage of corn attractive for oviposition, and not by a scarcity of moths. Apparently both these writers overestimated the relative abundance of eggs late in August and in September. The explanation by Phillips and Barber (10) that corn planted in midseason (May 8-29), which silks the latter part of July, usually receives the fewest eggs because it silks between the appearance of the first and second broods of moths does not seem valid. It is obvious, from observations in northern Virginia, that moths of the second brood would normally be abundant at Charlottesville during the latter part of July, and that the comparative abundance of attractive corn is a more important factor in the relative amounts of damage in field corn planted at different dates than has been realized. The conclusion by Phillips and Barber (12) that in the latitude of Virginia corn should be planted on or before May 8 to reduce the earworm injury is undoubtedly correct. According to the data obtained in the vicinity of the District of Columbia, it appears certain that the greater the acreage planted early in May the lower will be the percentage of infestation in such plantings.

SEASONAL DAMAGE

Phillips and Barber (11) found that, at Charlottesville and Richmond, Va., field corn planted between April 30 and May 15 showed less loss in yield due to the corn earworm than corn planted later. They considered it inadvisable to plant field corn in that section after the middle of May. Since their data in regard to injury in corn planted on different dates (11, table 4) give some idea of the seasonal abundance of the corn earworm in the two areas, they have been adapted for this purpose in table 7 and figure 4. Table 7 shows that slightly over half of the corn acreage included in their study was planted between May 1 and 15, and that the fields planted during this period had the smallest average number of kernels injured per ear as well as the lowest percentage loss in yield by weight. By adjusting the damage according to the acreage of corn planted during the various periods, it was found that the percentage of the total loss in yield was greatest in this period. The corn planted between June 1 and 15, amounting to 7.9 percent of the total acreage under observation, had approximately $2\frac{1}{2}$ times as many injured kernels per ear as

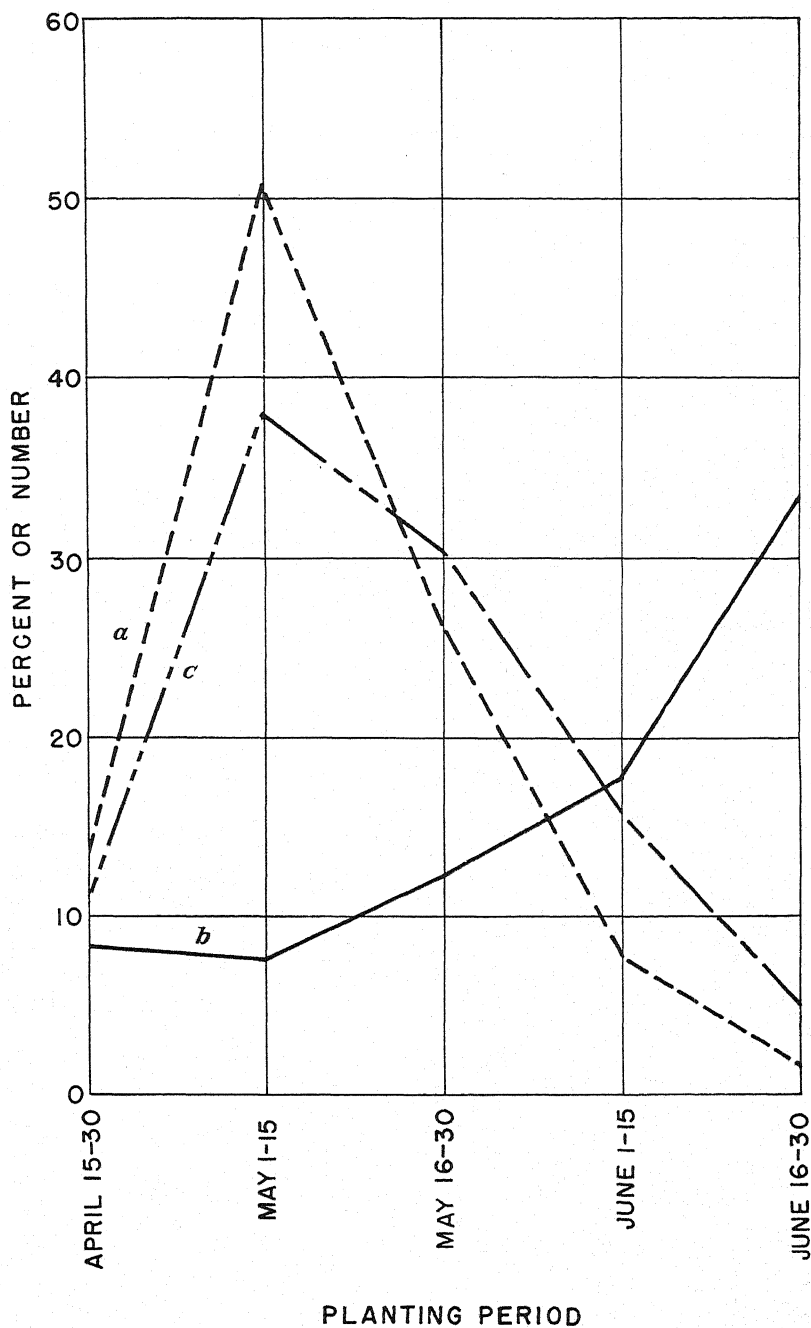


FIGURE 4.—Relation of acreage to the total loss in yield and seasonal kernel injury, Charlottesville and Richmond, Va., 1927 and 1928. (Adapted from data of Phillips and Barber (11)): *a*, Percentage of total acreage planted; *b*, kernels injured per ear; *c*, percentage of total loss in yield.

that planted between May 1 and 15, but the percentage of the total loss in yield by weight was less than half as much. Corn planted between June 16 and 30, 1.5 percent of the total acreage, was most severely injured, yet it bore only 5 percent of the total loss in yield by weight. Nearly 50 percent of the loss in yield occurred in the group of fields planted before May 16. The slight reduction in kernel injury in the fields planted May 1-15 under that in fields planted April 15-30 was apparently the result of a rapid increase in the acreage of corn attractive for oviposition. These data indicate that the latter part of the first brood and the second brood are of primary importance in the losses caused by the earworm. The statement by McColloch (9) that the first and second broods are of little importance in comparison with the third is not valid for the areas studied in Virginia.

TABLE 7.—Seasonal injury caused by the corn earworm at Charlottesville and Richmond, Va., in 1927 and 1928

(Adapted from Phillips and Barber (11, table 4))

Planting period	Approximate acreage planted	Portion of total acreage	Kernels injured per ear	Numerical rating of kernels injured ¹	Portion of total kernels injured	Reduction in yield by weight	Numerical rating of reduction in yield ²	Portion of total loss in yield
	<i>Acres</i>	<i>Percent</i>	<i>Number</i>		<i>Percent</i>	<i>Percent</i>		<i>Percent</i>
April 15-30.....	247	13.5	8.44	113.9	11.2	1.13	15.3	10.7
May 1-15.....	929	50.9	7.80	397.0	39.0	1.06	54.0	37.8
May 16-30.....	478	26.2	12.30	315.2	31.0	1.66	43.5	30.5
June 1-15.....	144	7.9	17.93	141.6	13.9	2.90	22.9	16.0
June 16-30.....	27	1.5	33.53	50.3	4.9	4.74	7.1	5.0
Total.....	1,825	100.0	-----	1,018.0	100.0	-----	142.8	100.0

¹ Calculated by multiplying the percentage acreage planted by the kernels injured per ear.

² Calculated by multiplying the percentage acreage planted by reduction in yield by weight.

Although the loss in yield caused by the earworm in late-maturing corn is less than in the more numerous early-maturing fields, the larval population is of primary importance in contributing to the population of overwintering pupae. A large percentage of the pupae that hibernate successfully under normal winter temperatures and precipitation enter the soil from such fields the latter part of August or the early part of September.

SEASONAL ABUNDANCE OF THE CORN EARWORM IN RELATION TO CLIMATE

The study of winter survival of the corn earworm in relation to its general abundance the subsequent season was begun in the fall of 1933. Hibernation cages containing larvae collected from field corn were established early in September each year except 1934, when inclement weather delayed their establishment until late in September. Experience had shown that there was a much greater mortality among individuals going into hibernation the latter part of September than among those entering the soil early in the month. This fact was clearly brought out by examination of field plots in the spring of 1935. Survival in these plots, where larvae began entering the soil during the third week of August, was 68 percent as compared with 4 percent in

the cages. Consequently the survival of pupae in cages in the spring of 1935 is not considered comparable with that in 1934, 1936, and 1937.

The most important factors affecting winter mortality of corn earworm pupae are temperature, soil moisture, and the presence or absence of snow cover during periods of low temperature. The vital temperatures in this area are those occurring in December, January, and February. The pupae are most susceptible to below-freezing temperatures when the soil moisture is high.

The climatological data presented in table 8 are condensed from the records of the Weather Bureau station at Washington, D. C., compiled by Weeks (14). The effect of weather conditions on pupal survival and the abundance of the corn earworm in the subsequent season is readily seen. During the winter of 1933-34 both the temperatures and the precipitation were subnormal. The pupal survival in hibernation cages was 14 percent, and the ear infestation in 1934 was 28.5 percent. In the winter of 1934-35 temperatures and precipitation were nearly normal, data from outdoor plots indicated a higher pupal survival, and the percentage of infestation in field corn in 1935 was 30.0 percent. In the winter of 1935-36 an accumulated temperature deficiency of 13.1° F. and an excess of 3.82 inches of precipitation resulted in a pupal survival of only 1 percent and an ear infestation of 24.1 percent in 1936, the lowest for the 4 years.

The effect on hibernating pupae of low temperatures when combined with a high soil-moisture content without protection by snow cover is brought out strikingly in the data for that winter. During January heavy rains shortly before a rapid drop in temperature, which continued at a low point for approximately 1 month, caused the soil to heave severely, and when the soil settled again in the spring it embedded a large percentage of the few surviving pupae, preventing the emergence of moths. The minimum temperature recorded in soil without vegetation that winter was 22.5° F. at a depth of 4 inches.

TABLE 8.—*Relation of winter temperatures and precipitation to the spring survival of pupae and the abundance of the corn earworm on field corn the subsequent summer in the area of Maryland and Virginia adjacent to the District of Columbia*

Season	Temperature Dec. 1 to Mar. 1			Precipitation Oct. 1 to Apr. 1		Pupal survival	Abundance of corn earworm	Fields less than 30-per-cent infested
	Mean	Accumulated departure from mean	Days below 20° F.	Total	Accumulated departure from normal mean			
	°F.	°F.	Number	Inches	Inches	Percent	Percent	Percent
1933-34.....	34.0	-3.2	29	15.31	-3.79	14.0	28.5	57.1
1934-35.....	35.4	+7	16	18.27	-.83	4.0	30.0	56.4
1935-36.....	30.7	-13.1	38	23.01	+3.82	1.0	21.1	72.5
1936-37.....	40.1	+14.9	1	20.35	+1.25	24.5	51.2	21.4

The mildest winter weather occurred in 1936-37, when the accumulated temperatures were 14.9° F. above normal and the precipitation was 1.25 inches above normal. This resulted in a pupal survival in cages of 24.5 percent and an ear infestation of 54.2 percent during the summer of 1937.

The most severe temperatures in any 1 month of the 4-year period were in February 1934, when the minimum temperature was -6.0°F . with a departure from the normal mean of -10.7° . The high survival of pupae in the spring of 1934 is attributed to the fact that the soil moisture was low because of a deficiency in precipitation from October 1 to April 1, and also to an adequate snow cover during the exceedingly low temperatures in February.

The importance of soil moisture as a factor in winter mortality of pupae was well brought out in outdoor insectary tests during the

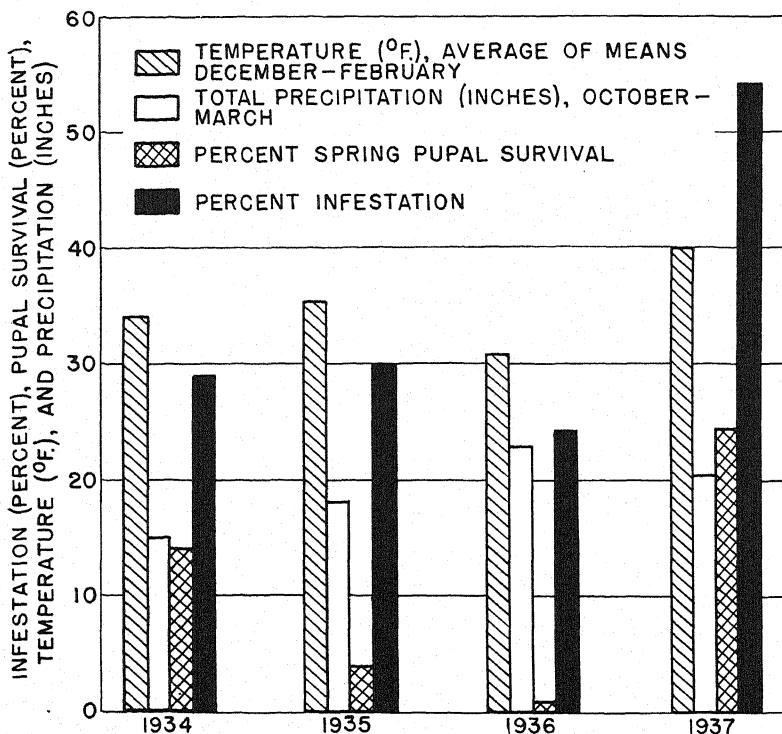


FIGURE 5.—Relation of mean temperature of December, January, and February and precipitation from October to April to spring survival of pupae and the abundance of the corn earworm the subsequent season.

winters of 1936-37 and 1937-38. These tests indicated that high percentages of the hibernating pupae can survive exposure to air temperatures as low as 14°F . if the soil is dry, whereas such low temperatures cause a high mortality if the soil is wet.

The relation of the average minimum temperature for December, January, and February and the precipitation from October 1 to April 1 to the spring survival of pupae and the percentage of earworm infestation the subsequent season is plotted in figure 5. There is a striking positive correlation between the average mean temperatures for the 3-month period indicated and the percentage of earworm infestation the following season. Except for 1935, when the results of the survival in hibernation cages were not considered comparable for reasons already explained, there is a positive correlation between the extent of

pupal survival and the average mean temperature for the 3 winter months.

A high percentage of soil moisture contributes substantially to pupal mortality when not in combination with low temperatures. The prepupae and the newly-formed pupae are particularly susceptible in wet soil. Disease organisms, especially entomogenous fungi, are more numerous and effective in soils high in humus content, and if the soil is not too acid the common species of earthworms are abundant. Heavy rains bring the earthworms to the surface, where they invade the pupal burrows, and many earworm pupae become embedded in their castings. Not only is there a high mortality among pupae thus embedded, but the breaking down of the burrows prevents many moths from escaping to the surface.

The data obtained in this study indicate that when the mean temperature for December, January, and February is about 35° F. and precipitation is normal, the corn earworm will be sufficiently abundant to cause material damage to the corn crop. It appears safe to predict that when, with normal precipitation, the mean temperature for December, January, and February drops to 30° or below, the abundance of the corn earworm the following season will be considerably below normal.

SUMMARY

A study has been made of the general seasonal abundance of the corn earworm (*Heliothis armigera* (Hbn.)) in central Virginia and in the area in Virginia and Maryland adjacent to the District of Columbia, and also of the climatic factors that influence the seasonal abundance from year to year. This study has been made not only in experimental plantings but also by surveys of populations in corn-fields.

At Charlottesville, Va., in 1932, the maximum number of eggs per silk in separated seasonal plantings of sweet corn was found in September, the minimum number in August, and the number between these extremes in the latter part of July. The percentage of ears infested by larvae in these plantings showed a similar trend. The number of eggs per silk and the percentage of ear infestation were at a minimum when general observations indicated that field corn attractive for oviposition was at its peak.

In 1934 and 1935 the percentage of infestation in seasonal plantings of sweet corn in a single block at Arlington, Va., showed a trend similar to that observed at Charlottesville. The infestations were much higher than those found in a field survey. This condition is attributed to a concentration of ovipositional activity on contiguous early-silking plots and a building up of an infestation in the later silking plots by succeeding generations. Such seasonal plantings are consequently not considered to give a satisfactory index of the general seasonal abundance of the corn earworm.

In 1936 at Arlington the number of eggs per silk was at a minimum when the acreage of corn in silk attractive for oviposition was at its maximum. When adjusted to the acreage attractive for oviposition, the population of eggs was near its height at the same time that the acreage of corn most attractive for oviposition was at its maximum. The data of 1936 indicate that the moth population reached its maximum the latter part of August, and that the heavy infestation on late-

maturing corn was the result of a concentration of oviposition on a rapidly declining acreage of attractive corn while the population of moths was still high.

In northern Virginia the corn earworm hibernates in the pupal stage more or less successfully. Emergence of moths from overwintering pupae begins the latter part of May and continues until about July 15. The first eggs are usually found the last of May. Moths of the second brood begin to appear the first week of July. Because of this overlapping of generations the number of generations can only be estimated. There are probably two complete generations and in normal seasons a considerable part of a third generation.

Most of the pupae that are to overwinter successfully become established in the soil between the middle of August and the middle of September; therefore larvae developing from eggs deposited after the first week of September are of little significance in providing overwintering populations in this area.

In surveys of field corn in Montgomery County, Md., and in Fairfax County, Va., during the period 1934-37 the percentage of ears infested was not an accurate measure of the seasonal abundance of the corn earworm. The slightly higher percentage of infestation in the few early-silking fields was due to a concentration of oviposition by the available moth population on such corn. With the rapid increase in corn acreage attractive for oviposition, the percentage of infestation receded slightly, although the population was actually increasing rapidly. The larval population reached its maximum about the time when corn in fresh silk was at its maximum. The highest percentage of infestation occurred in the relatively small acreage of late-maturing corn and was due to concentration of ovipositional activity. Cannibalism causes a heavy larval mortality in late-maturing corn and reduces the accuracy of egg and moth population determinations in such corn about 30 days after it has begun to silk. The method used to determine seasonal abundance consequently gives an index of the number of larvae entering the soil for pupation. The best method of determining the seasonal abundance of moths is to adjust the egg population per silk according to the amount of corn in silk attractive for oviposition.

In a study of the seasonal damage caused by the corn earworm Phillips and Barber (11), found that the percentage of loss by weight was lightest in corn planted before May 16. The corn planted during this period accounted for about two-thirds of the total acreage and about half of the total loss in yield. This analysis showed that the loss in yield in the small acreage of late-planted corn is of minor importance. The late-planted fields are of major importance in providing overwintering pupae. The recommendation that corn be planted as early as compatible with obtaining a satisfactory stand has further justification because the greater the acreage planted at such time, the lower is likely to be the percentage of ears infested.

During the period 1934-37 there was a positive correlation between the average mean temperature for December, January, and February, the rate of survival of overwintering pupae, and the abundance of the corn earworm in field corn the following season. In 1936 low temperatures and high soil moisture with inadequate snow cover resulted in a heavy mortality of hibernating pupae and the lowest

population for the 4-year period. In an open insectary a high percentage of pupae survived an exposure to 14° F. on dry soil, whereas a heavy mortality followed this exposure on moist soil. In the area studied, when winter temperatures and precipitation are normal there is sufficient survival to cause material damage to the corn crop in the subsequent season. When the mean winter temperature falls to 30° F. or below, a reduction in the abundance of the corn earworm in the field may be expected.

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YELLOW DWARF OF POTATO IN WISCONSIN¹

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INTRODUCTION

During the growing seasons of 1930, 1931, and 1932 heavy losses from disease were sustained in the potato crop in central Wisconsin, particularly in Portage, Waupaca, and Waushara Counties. One of the outstanding characteristics of those epidemics was the general prevalence of poor stands from apparently sound tubers. Investigations beginning in 1933 have shown that the yellow dwarf virus was the primary cause of the difficulty. In 1935 the disease was destructive in Washington County, in the southeastern part of the State, many fields there being close to a total loss. In 1937 it was more widely prevalent and destructive in Wisconsin than in any year on record, being common not only in central Wisconsin but throughout the eastern part of the State, particularly in Outagamie, Fond du Lac, and Washington Counties. This paper is a report of field and greenhouse studies of the disease which have been under way during the period from 1933 to 1938, inclusive.

SYMPTOMS OF YELLOW DWARF IN WISCONSIN

In general the symptoms of the disease in Wisconsin coincide with the first description by Barrus and Chupp (1)² from New York and with that by Muncie (7) from Michigan. Certain differences prevail, however, which may be expected under the influence of environment. Goss and Peltier (3) showed that in greenhouse-grown plants infected with the yellow dwarf virus severe disease development occurred at 25° C., while at 15° there was only very slight internal necrosis of the tubers and no foliage symptoms. In central and southern Wisconsin the disease appears on the early-planted crop in late May or early June. In the main crop, which is planted from May 25 to June 20, the disease is commonly seen on young shoots soon after emergence.

SECONDARY TOP SYMPTOMS

The secondary symptoms on the foliage, i. e., those on plants from infected seed pieces, are the most striking. In a favorable environment the shoots remain dwarfed and the internodes shortened. The leaflet margins roll upward while the long axis of the leaflet curves downward, particularly at the apex. Thus the plant is often reduced to a rosette (fig. 1, *B*). Both stem and leaves take on a yellow cast while the upper surface of the latter becomes slightly rugose. The change in color is often evident first at the growing tip of the plant (fig. 1, *C* and *D*), but it may come on almost simultaneously over the

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² Italic numbers in parentheses refer to Literature Cited, p. 279.

entire plant if infection has come from a diseased seed piece. Secondly infected plants usually produce only small misshapen tubers, commonly on short stolons. Prompt dieback from the tip has been described by Barrus and Chupp (1) and by Muncie (7). This does not necessarily follow under Wisconsin conditions, at least not promptly. Some infected plants die within a few days to 2 weeks after emergence; many survive for longer periods. The course of the disease at this stage is influenced by temperature and moisture. High temperature and low humidity tend to hasten the death of infected plants.

Occasional plants assume a rosette appearance early without any marked change from normal color. They remain stunted throughout the growing season but with little or no top necrosis. These symptoms, which are referred to tentatively as "green dwarf," are commonly found in fields where typical yellow dwarf symptoms occur. Whether or not they represent a phase of yellow dwarf or a distinct disease has not been determined.

Pith necrosis of stems is a common feature. It appears shortly after chlorosis of the foliage and, beginning near the growing tip, may eventually extend the entire length of the main stem. It may or may not be most severe at the nodes. As yellow dwarf plants become weakened, the vascular system of the lower portion of the stem may turn brown and the fibrous roots become discolored and shriveled. At this stage the disease may readily be confused with certain stages of one or another of the fusarium wilts. In all probability secondary soil organisms are contributing causes to these symptoms through their invasion of the root system of plants weakened by yellow dwarf.

Often the most common secondary symptom of yellow dwarf under central-Wisconsin conditions is failure of the infected seed piece to produce an emerging plant. In warm soil many seed pieces do not germinate at all; others produce shoots that die before they reach the surface. When environmental conditions are such as to favor non-emergence the lack of stand is a much more striking sign of the disease than the symptoms on the small percentage of infected plants which emerge. Infected seed pieces from emerging and nonemerging hills are commonly hard and glassy in texture. They usually remain intact without decay until late in the growing season or until harvest.

The symptoms discussed up to this point are those that appear promptly on plants from infected seed pieces under favorable environment. In some cases delayed secondary symptoms appear under the same environmental conditions and ordinarily they are not distinguishable from primary symptoms resulting from spread during the current season. By making experimental plantings on the tuber-unit basis, usually with three hills from each seed tuber, late secondary symptoms have been distinguished from primary symptoms by the fact that the three hills in a given unit usually succumb simultaneously in the case of the former type, while in the latter type the symptoms are not correlated with hill units. Even under conditions favorable for disease expression the first appearance of the delayed symptoms may be 6 weeks or more after emergence, and often after the plants have grown quite normally to nearly maximum height. In fact, there is reason to believe that some plants infected from diseased seed pieces fail to show symptoms during the entire second season.



FIGURE 1.—Secondary top symptoms of yellow dwarf on potato plants. All are of the same age and grew in the same field of Irish Cobbler variety planted in the latter part of April 1938, in central Wisconsin; photographed July 7. *A*, Healthy plant. *B*, Plant that became dwarfed early, most of the leaflets showing rugosity, upward roll of margins, and downward curve of the longitudinal axis. *C*, Plant in which several leaves developed to normal size before acute symptoms appeared in the youngest leaves. Rugosity and slight marginal roll in the older leaves are noticeable when comparison is made with healthy plant in *A*. *D*, Plant that grew normally for a still longer period than the one in *C* before secondary symptoms began to appear in the youngest leaves, where the leaflet margins are beginning to roll upward while the downward longitudinal curving has just been initiated. It will be seen that the leaflets of most of the lower leaves are normal as to size, margin, and smoothness of lamina when compared with the healthy plant, *A*. Blossoming in all diseased plants is noticeably suppressed.

The first signs are slight chlorosis and marginal upward rolling of leaflets near the growing tip, and in this respect they are not unlike primary symptoms in appearance. Usually, however, chlorosis of the entire plant comes on more promptly than after primary infection and, in the main, all shoots from an infected seed piece show the symptoms simultaneously. Occasionally a healthy and an infected shoot arise from the same seed piece. Such healthy-appearing shoots commonly develop delayed symptoms; rarely do they remain healthy in appearance until harvest. When seed tubers are cut and planted on the hill-unit basis occasional cases arise in which one hill of a unit remains healthy and produces tubers free from yellow dwarf while the others produce diseased plants and tubers. Quite as often as not the stem-end piece of a unit produces a healthy hill while the bud-end pieces of the same tuber produce diseased hills. This indicates that as the virus progresses through the stolon it does not necessarily affect the nearest eyes and may become distributed irregularly in the tissues.

PRIMARY TOP SYMPTOMS

The primary symptoms on foliage arising from current-season dissemination of the virus appear as chlorosis and upward roll of leaflets in the vicinity of the growing point. Symptoms of the disease develop progressively downward and pith necrosis, appearing first at the tip, follows down the stem. Often only a single shoot of a hill is affected and symptoms in the tuber are usually confined, in such a case, to those arising from the diseased shoot. It has been definitely established by study of clonal progenies from selected hills that many plants become infected, presumably late in the growing season, and produce tubers infected with the virus, although they show signs of the disease on neither tops nor tubers.

TUBER SYMPTOMS

The effect of yellow dwarf on the tubers varies widely. Early-diseased plants commonly produce small misshapen tubers, which in cross section show small necrotic areas usually scattered throughout the pith and internal phloem. Tubers from plants showing primary symptoms or delayed secondary symptoms are reduced in size and are necrotic internally. Growth cracks are common, but, as they result from many other causes, they should not be considered as a diagnostic character of yellow dwarf. Internal necrosis of tubers affected with yellow dwarf is not typically a net necrosis and should not be confused with the latter type of internal discoloration. Since the yellow dwarf necrosis occurs very commonly on the same soils and in the same regions in Wisconsin as the nonparasitic internal brown spot or sprain, the two types of tuber necrosis may be easily confused. In fact, where symptoms of the latter disease are diffused and scattered in the tissue, the two cannot be distinguished in the tuber (fig. 2.).

Unlike the potato leaf roll disease, in which necrosis is confined to the phloem, the necrotic tissue in stems and tubers of yellow-dwarf plants may be found in the pith, the inner phloem, and to some extent in the outer phloem and in the cortex. Xylem elements are seldom affected. In sections of fresh tissue the discolored areas appear as groups of disorganized cells in which has accumulated

amorphous, amber-colored material. The latter stains heavily in fixed sections when coal-tar dyes or haematoxylin are applied.

SUMMARY OF DIAGNOSTIC FEATURES

In view of the various symptoms of yellow dwarf that overlap those of other potato diseases it is obvious that no single one can serve as an infallible guide for diagnosis of this disease. This is particularly true under environmental conditions in which full expression is prevented and the disease is partially masked. Under favorable conditions the stunting, the upward marginal roll and the downward curving along the longitudinal axes of the leaflets, the chlorosis beginning at the growing tips followed by pith necrosis of the stem,

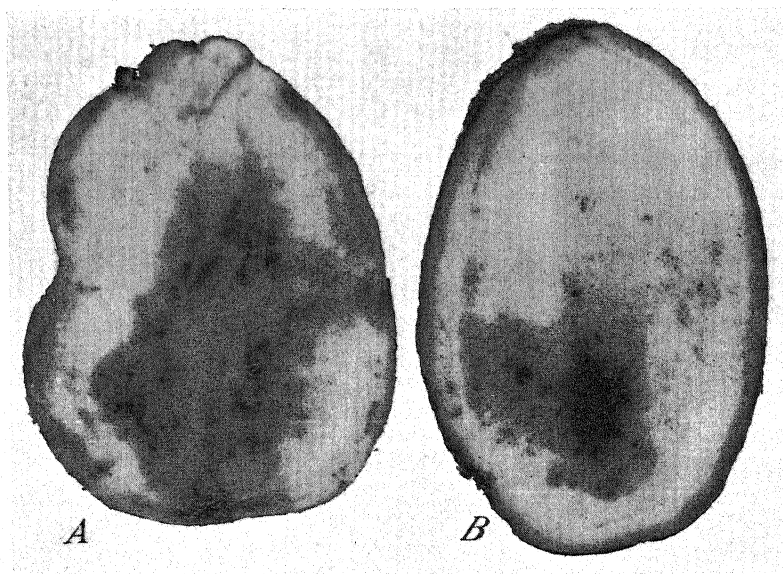


FIGURE 2.—*A*, Symptoms of yellow dwarf in potato tuber; necrosis occurs in small areas throughout the pith and internal phloem. *B*, Internal brown spot or sprain. This disease occurs commonly in tubers grown on sandy soils in central Wisconsin, is very similar to yellow dwarf, and sometimes is indistinguishable from it.

and the malformation and necrosis of the tubers, taken together, afford a very satisfactory basis for differentiation of yellow dwarf from other maladies. It should be emphasized, however, that, particularly in late-season spread, infected plants may mature with no signs of the disease either on top or tuber.

RELATION OF YELLOW DWARF TO POOR STAND

Inasmuch as the yellow dwarf epidemics in central Wisconsin in 1931 and 1932 were characterized primarily by lack of stand, a study was made in 1933, 1934, and 1935 of the relation of nonemergence to yellow dwarf.

In 1935, 15 lots of tubers designated as group 1, were taken from the field run of the poor-stand area of 1932; 8 lots (group 2) were

taken from fields of relatively good stand on the fringe of the poor-stand areas; 7 lots of tubers (group 3) were from certified fields of northern Wisconsin. Forty tubers from each lot were planted—20 on June 6, and 20 on June 23—at Almond, Portage County, in the region where disease losses had previously been very heavy. The tubers were cut into three seed pieces each, the stem-end half of the tuber being cut first and the bud half divided into the second and third pieces. These were planted consecutively in units of three hills per tuber.

TABLE 1.—Occurrence of nonemerging hills and hills with plants showing secondary symptoms of yellow dwarf in planting made in 1933 from various seed-tuber lots at Almond, Portage County, Wis.

Source of seed lots	Lot No.	Planting on June 6		Planting on June 23	
		Nonemerging hills	Hills showing yellow dwarf	Nonemerging hills	Hills showing yellow dwarf
		Percent	Percent	Percent	Percent
Group 1.—Lots from the field run of fields in the poor-stand area of 1932.	1	87	3	85	3
	2	81	2	77	6
	3	74	3	78	3
	4	49	3	77	6
	5	48	5	62	3
	6	44	3	41	4
	7	37	3	49	3
	8	33	2	39	1
	9	28	3	37	1
	10	27	2	28	3
	11	15	6	33	4
	12	15	0	14	3
	13	15	6	16	4
	14	13	0	20	3
	15	2	2	9	0
Average.....	1-15	38	3	44	3
Group 2.—Lots from good fields on the fringe of the poor-stand area in 1932.	16	23	0	20	0
	17	6	0	4	0
	18	5	0	3	0
	19	5	0	3	0
	20	3	0	7	0
	21	3	0	20	0
	22	1	0	14	0
	23	0	0	3	0
Average.....	16-23	6	0	9	0
Group 3.—Lots from certified fields of northern Wisconsin.	24	3	0	2	0
	25	1	0	2	0
	26	1	0	3	0
	27	1	0	2	0
	28	1	0	2	0
	29	0	0	0	0
	30	0	0	0	0
Average.....	24-30	1	0	1	0

The final data from the 1933 plot are recorded in table 1. The lots in group 1, coming from the heavily diseased area of 1932, plants generally failed to emerge in a high percentage of the hills. With the exception of lot 15, in which the stand was good, missing hills ranged from 13 to 87 percent. In the entire group 38 percent were missing in the first planting and 44 percent in the second planting. The percentage of plants showing secondary yellow dwarf symptoms was consistently low. In no case did more than 6 percent of the hills contain plants showing the disease, while the total for group 1

was 3 percent of diseased plants in each of the plantings. In group 2 no plants were observed showing secondary yellow dwarf. The missing hills were usually few in number, and the percentage for the group as a whole was much less than in group 1. In the lots from certified seed the stand was excellent and no yellow dwarf was observed. A view of this plot is given in figure 3.

The close association of severe losses due to poor stand and the occurrence of secondary yellow dwarf evident in group 1 was still more striking when individual tuber units were studied. In general, yellow dwarf hills occurred most commonly in units with missing hills. Many yellow dwarf plants died soon after emergence and by mid-



FIGURE 3.—The effect of yellow dwarf upon stand. View of the 1933 experimental plot at Almond, Wis., in which seed tubers from various sources were compared. In the center are two rows planted with seed from a yellow dwarf field of 1932 from which a very low percentage of emergence was secured. At the right are two rows from certified seed from northern Wisconsin, which yielded a 98-percent stand.

season the percentage of missing hills in group 1 had increased considerably. The occurrence of units with two missing hills and one yellow dwarf hill, or one missing hill and two yellow dwarf hills was the usual case. In groups 2 and 3, on the other hand, the missing hills were scattered irregularly, usually singly in a unit of three hills.

Hills which appeared to remain healthy throughout the season were selected from four lots in group 1, one lot in group 2, and seven lots in group 3. These were planted in 1934 in two locations—at Madison on May 28 and at Almond on June 11. Seed tubers from certified fields in northern Wisconsin were included in each planting. The plantings at the two locations yielded closely similar results. The lots from group 1 and 2 are treated collectively in the data in table 2. It is quite evident that the virus had been transmitted to many of the

hills in the previous season too late to have caused symptoms of yellow dwarf. The percentage of yellow dwarf plants averaged higher than in the parent lots in 1933. The percentage of missing hills, though not as high as in 1933, was nevertheless of the same relative order. In the five lots from group 3 of 1933 (table 1) some contamination by the virus had occurred, since the percentage of disease had increased from none in 1933 to 2 and 4 percent at Almond and Madison, respectively, in 1934, and the loss in stand increased slightly over that of 1933.

TABLE 2.—Occurrence of nonemerging hills and hills with plants showing secondary symptoms of yellow dwarf in 1934, planting at Almond, Portage County, and at Madison, Wis., from healthy-appearing plants at Almond in 1933

1933 lot No.	1934 lot No.	Planting at Almond, June 11		Planting at Madison, May 28	
		Non-emerging hills	Hills showing yellow dwarf	Non-emerging hills	Hills showing yellow dwarf
		Percent	Percent	Percent	Percent
4.....	1	20	7	27	10
5.....	2	32	13	45	2
7.....	3	13	12	12	10
11.....	4	33	7	27	7
22.....	5	23	3	8	12
Average lots.....	1-5	24	8	24	8
24.....	6	10	8	2	3
26.....	7	6	3	7	2
27.....	8	0	1	8	0
29.....	9	3	0	2	0
30.....	10	6	1	3	13
Average lots.....	6-10	5	2	4	4
Certified seed tubers from northern Wisconsin.....	1	1	1	2	0
Seed tubers from yellow dwarf hills of 1933.....		53	17		

In the process of selecting apparently healthy hills in rows of group 1 and group 3 in 1933 it was obvious that more of the former selected at random carried the virus than did the latter. This cannot be explained satisfactorily on the basis of the hills in group 1 being exposed more openly to nearby yellow dwarf plants than were those in group 3, since the various lots were placed in randomized order in the plot of 1933. From this and the cases studied subsequently it is suggested that some plants carry the virus through the second season without showing symptoms. A lot exposed to a heavy infestation may possibly produce more symptomless carriers at the end of the next season than a less heavily infected lot. This point deserves further study.

Tubers from representative healthy hills were selected in the autumn of 1934 from lots No. 7 and 11 (table 2) at both Almond and Madison. These were planted at Almond on June 5, 1935. The general result was that there were very few missing hills. On the other hand, a much larger percentage of yellow dwarf plants was noted than in 1933 and 1934. During the growing seasons of 1934 and 1935 continuous records of air temperature were kept. In 1933 and 1934 the air temperature was above normal in June while in 1935 it was somewhat below normal. In table 3 the summary of nonemergence and

yellow dwarf for the three seasons is given, as well as the bihourly mean of the air temperature for the 21-day period after planting in the last two seasons. It is to be seen that the average temperature was approximately 7° C. lower in 1935 than in 1934. It is also to be noted that the totals of missing hills and yellow dwarf hills in each year are close to one another. In the cool year the percentage of nonemergence was low and that of yellow dwarf symptoms was high, while in the warm year the opposite was true.

The close correlation of poor stand with yellow dwarf symptoms during the field studies of 1933, 1934, and 1935 pointed definitely to the probability that they were phases of the same disease. It was also indicated that environment played an important part in determining which phase of the disease predominated. Further studies were therefore directed toward the relation of environment to the expression of yellow dwarf symptoms.

TABLE 3.—*Comparison of relative amounts of nonemergence and secondary yellow dwarf hills at Almond, Wis., during 1933, 1934, and 1935*

Year	Mean temperature ¹	Hills planted	Missing hills	Yellow dwarf hills
	° C.	Number	Percent	Percent
1933	22.8	3,600	41	3
1934	22.8	600	24	8
1935	16.0	653	1	29

¹ Bihourly mean of air temperature for a period of 21 days after planting.

RELATION OF ENVIRONMENT TO YELLOW DWARF

FIELD EXPERIMENTS

In 1933 a quantity of tubers was collected from yellow dwarf hills in the plot at Almond. In 1934 some were planted at Madison in the southern part of the State, some were planted at Almond in the central part, and the remainder were planted at Ashland in the northernmost part. These locations were selected to provide as wide a range of temperature conditions during the growing season as might be secured within the State. Two plantings were made at each location, the first on or about May 15, the second about June 1. The differences between the results of the two plantings in any given area were not great. They are therefore combined for each location in table 4. As the season progressed the spread between the temperatures at Madison and Almond was not large; the Ashland area was decidedly cooler. In the first two locations, where temperatures were relatively high following planting, the percentage of missing hills was high and that of yellow dwarf comparatively low. In the northern location more than 90 percent of the plants emerged and nearly 50 percent showed symptoms of yellow dwarf. As in the case of the results already shown in table 3, it was evident that in the northern location where lower temperatures prevailed a greater percentage of plants emerged. The temperature was relatively high following planting at both Almond and Madison and there was little difference between results from these locations.

TABLE 4.—*Comparison of emergence and yellow dwarf in southern, central, and northern Wisconsin, 1934*

Station	Relative location in Wisconsin	Total hills	Non-emerging hills	Yellow dwarf hills
		<i>Number</i>	<i>Percent</i>	<i>Percent</i>
Madison.....	Southern.....	48	52	21
Almond.....	Central.....	48	46	21
Ashland.....	Northern.....	47	9	49

GREENHOUSE EXPERIMENTS

The study of the relation of air and soil temperature to the disease was continued under controlled conditions in the greenhouse. Seed tubers were selected from yellow dwarf-infected hills in the field and stored until they had passed the dormant period. In preparation for the experiments each tuber was divided into a sufficient number of seed pieces to provide a single piece for each of the air or soil temperatures being studied in a given series. Air-temperature studies were carried out in 10-inch clay pots placed in a series of greenhouses in which the temperature was kept very uniformly at 16°, 20°, 24°, and 28° C., respectively. In these cases the temperature of the soil was usually 2° to 3° lower than that of the surrounding air, owing to the cooling effect of evaporation of water from the pots. Soil-temperature studies were conducted in Wisconsin soil-temperature tanks, which were regulated to maintain the soil at 16°, 20°, 24°, and 28°, respectively. The plants growing in cans inserted in the temperature tanks were subjected to a common air temperature which fluctuated from 20° to 24°. In all cases control pots planted with seed pieces from known healthy stock were included.

AIR-TEMPERATURE STUDIES

The results of two experiments are reported in table 5. The first of these was conducted in the winter of 1934-35 and the second in the winter of 1935-36. There were uniformly good germination and growth in the controls in both cases and the data are, therefore, not included in the table. In the first experiment all seed pieces germinated; in the second there was a reduction of stand at the two highest temperatures. At 16° C. it was not possible to distinguish between plants from diseased and healthy seed pieces. When they were split longitudinally slight necrosis in the pith was noted in a small percentage of plants from diseased seed tubers. Slight external symptoms occurred at 20° and a few plants showed faint flecking in the pith. There was a marked contrast between the behavior of the plants grown at 24° and those grown at 20°, for symptoms occurred on most plants and internal pith necrosis occurred in all at the former temperature. The occurrence of pronounced external and internal signs of all plants which emerged at 28° showed that the clones were all infected by the virus. Since it may be expected that the plants growing at the lower temperatures had been infected in most instances, the suppression of symptoms was nearly complete up to 20°. These results are in general accord with those of Goss and Peltier (3).

TABLE 5.—*Effect of air temperature on development of yellow dwarf*

Experiment No.	Duration of experiment	Air temperature	Seed pieces planted	Non-germinating seed pieces	Plants showing external symptoms	Plants showing pith necrosis
		°C.	Number	Percent	Percent	Percent
1	Dec. 15, 1934, to Feb. 5, 1935, 52 days.....	16	20	0	0	10
		20	20	0	10	15
		24	20	0	85	100
		28	20	0	100	100
2	Feb. 3 to Mar. 21, 1936, 47 days.....	16	20	0	0	5
		20	20	0	5	15
		24	20	5	90	100
		28	20	25	100	100

SOIL-TEMPERATURE STUDIES

Several experiments were conducted at controlled constant soil temperatures during the winter periods of 1934-35 and 1935-36. As already indicated, the soil temperatures were held at 16°, 20°, 24°, and 28° C., while the air temperature fluctuated from 20° to 24°. The duration of each experiment was from 6 to 7 weeks. Control pots of healthy stock of the same variety as that of the diseased tubers were always included. Complete germination and consequent healthy plants were secured uniformly in the controls. The data from plantings of diseased tubers are summarized in table 6.

TABLE 6.—*Effect of various soil temperatures upon the emergence of plants grown from yellow dwarf-infected seed pieces of several varieties*

Variety	16° C.		20° C.		24° C.		28° C.	
	Seed pieces planted	Plants emerged	Seed pieces planted	Plants emerged	Seed pieces planted	Plants emerged	Seed pieces planted	Plants emerged
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Early Ohio.....	24	88	24	67	24	54	24	0
Irish Cobbler.....	18	100	18	72	18	11	18	6
Russet Rural.....	12	100	12	92	12	58	12	0
Rural New Yorker.....	18	83	18	61	18	6	18	6
Katahdin.....	36	100	36	86	36	58	36	0
Total.....	108	94	108	76	108	41	108	2

The trend of results was the same in each of the 5 varieties. There was a progressive decline in stand with increase in temperature. At 24° C. the average reduction was more than 50 percent while at 28° only 2 out of 108 seed pieces produced emerging shoots. These results agree with the field observations already reported, which indicated that high soil temperatures tended to reduce the stand and that nonemergence may be an important phase of the yellow dwarf disease. The proof of this contention is provided in this series of experiments, wherein a seed piece from each tuber clone was grown at each soil temperature. There is therefore no question that most, if not all, of the plants which emerged at the low temperatures were infected. Further demonstration of the fact that missing hills in yellow dwarf fields are usually the result of response to high soil-temperature effects on infected seed pieces was made in 1937. A large

number of such hills were dug 4 to 6 weeks after planting and generally the seed pieces were found to be about as turgid and free from decay as when they were planted. Many of these were collected and removed to the greenhouse where they were planted in soil held at 14° to 16° C. Practically all of them produced shoots that appeared to be quite as normal at this temperature as those from tubers of healthy stock that had been held over in storage from the previous autumn. When the temperature was increased to 24° yellow dwarf symptoms developed.

The influence of the various soil temperatures upon the occurrence of the disease in the tops is of interest. As already stated, the tops grown from tubers at the various soil temperatures were exposed to a common air temperature fluctuating from 20° to 24° C. From the results in the air-temperature series (table 5) this is an intermediate position between the most favorable air temperature for rapid disease development and that at which external symptoms are suppressed. A fully representative condition of the tops at the end of the experiments is shown in figures 4 and 5, which illustrate the Early Ohio and Russet Rural series, respectively, at the end of the experiments. The plants that emerged in 24° soil all remained dwarfed and showed symptoms promptly and decidedly. The air temperature was thus favorable in this case. In 20° soil the plants were somewhat dwarfed as compared with the controls at the same temperature, but there was clearly a decided depressive effect upon symptom development. In 16° soil there was no evidence of disease and the size of diseased and control plants was very nearly the same at the end of the experiments.

The soil-temperature series show that, whereas increase in soil temperature in which yellow dwarf-infected tubers are planted tends to reduce the percentage of emergence, decrease in soil temperature not only encourages emergence but it also tends to retard the development of top symptoms. It may be expected, therefore, that in areas with relatively cool growing seasons the field appearance of yellow dwarf would be less pronounced than in warmer regions. Slower development of secondary symptoms would be more common, while the masking of their appearance as well as those of primary infection would be greatly enhanced. Nonemerging hills would be less prevalent.

EPIDEMIOLOGY OF YELLOW DWARF

The incidence of this virus disease of potato has been extremely sporadic. While no season since 1933, when it was first distinguished in Wisconsin, has passed without its appearance in the central part of the State, its destructiveness has varied greatly from year to year. Where seed tubers from severely diseased fields of 1932 were used in 1933, losses were commonly heavy. In 1934 losses were much less severe and in 1935 and 1936 the stands were good and the percentage of diseased plants was relatively low. This period of 3 consecutive years with declining incidence of disease led to a return to the common practice of using locally grown seed in central Wisconsin. In 1937 another severe epidemic occurred which extended through the eastern half of Portage County in an eastern to southeastern direction as far as Washington County near Milwaukee. The nature of the disease

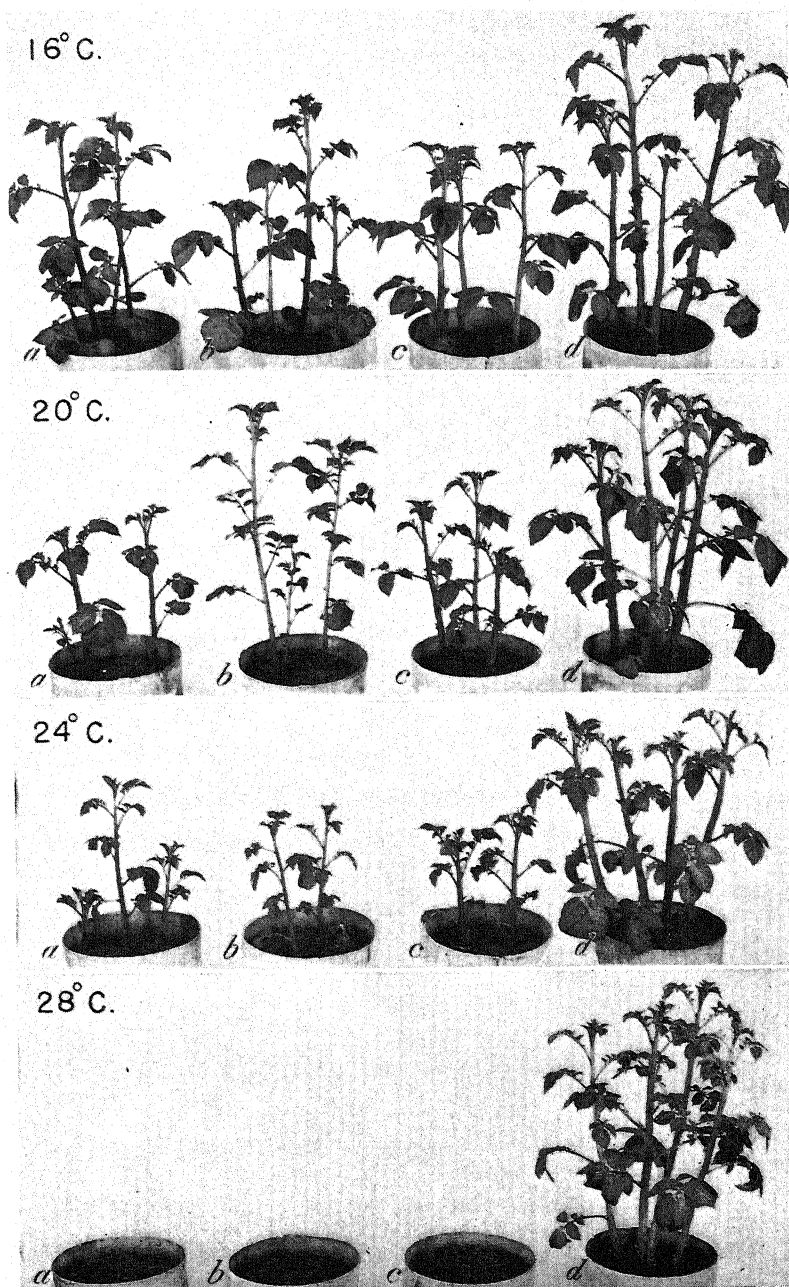


FIGURE 4.—Representative cans from the soil-temperature study with healthy (d) and yellow dwarf-infected seed tubers (a to c) of the Early Ohio variety. Each tuber used was divided equally between the four soil temperatures. Note reduction in stand at 24° and 28° and the suppression of aerial symptoms at 16° C.

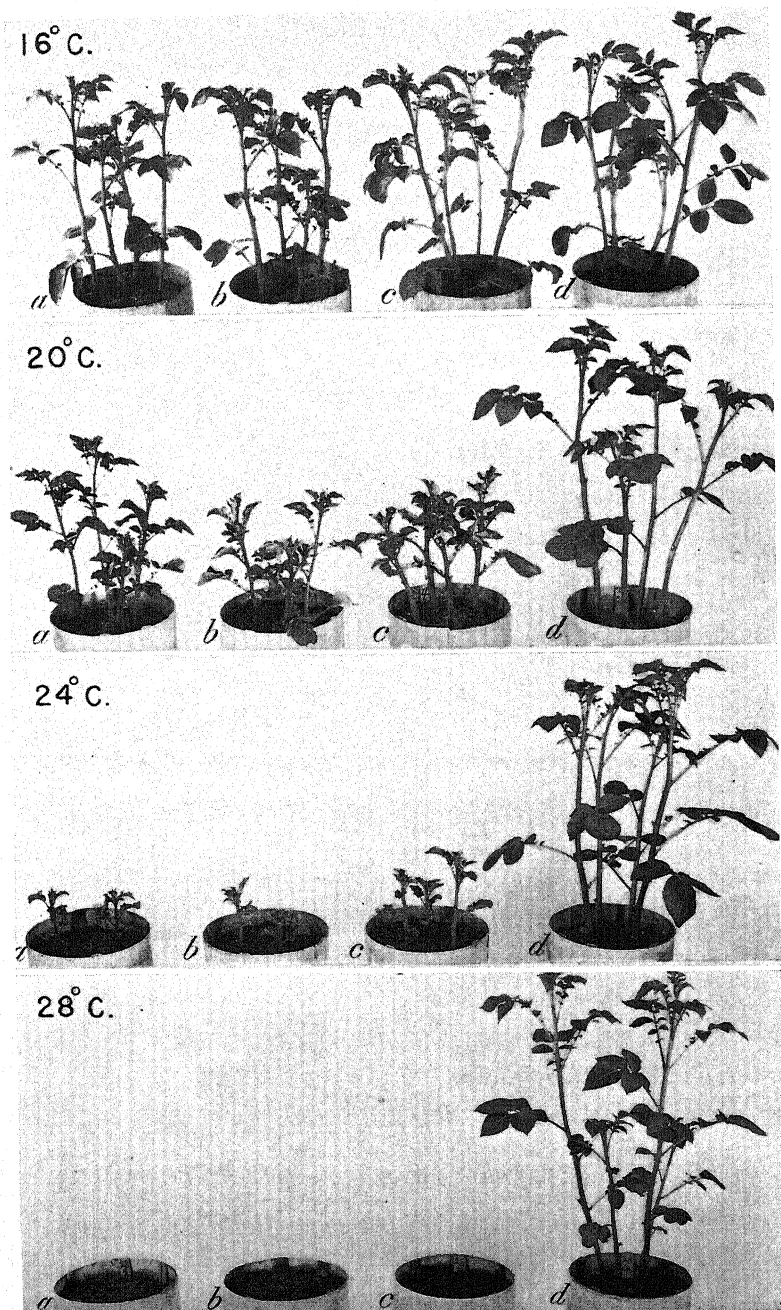


FIGURE 5.—Representative cans from the soil-temperature study with healthy (d) and yellow dwarf-infected seed tubers (a to c) of Russet Rural variety. Compare with figure 4.

development in that year, consisting of general reduction of stands and considerable percentages of early developing secondary symptoms, showed quite clearly that a general spread of the virus had occurred late enough in 1936 to provide infection without visible primary symptoms. A localized very severe epidemic had occurred in Washington County in 1935 in fields planted with local-grown seed tubers of 1934, although diseased plants were rare in that district in the latter year. In 1937 the development of current primary symptoms set in early in central Wisconsin and they continued to develop rapidly after August 1. Any seed tubers used from fields in the path of the 1937 epidemic produced typically poor stands in 1938. Exceptional losses were averted in the latter year by the general introduction of planting stocks from disease-free areas.

Periodic severity of yellow dwarf has been recorded also in Michigan and in New York. Muncie (6) pointed out that a field in Michigan contained 0.5 percent, none, and 24 percent of diseased plants in 1929, 1930, and 1931, respectively, although in each year seed tubers from the previous crop on the same field were used. In another case, when seed tubers from a 1930 field showing only 0.1 percent of yellow dwarf were planted in the same field in 1931, 50 percent of the plants were affected. It is significant that in both cases the epidemic development of 1931 followed a low-disease year in 1930. In New York, Taylor (8), in describing the development of yellow dwarf on nine varieties and strains of potato, reported a low disease incidence but apparently a serious seasonal spread in 1934, since the number of infected plants in the 1935 crop was as high as 48 percent in one lot. When tubers were saved from healthy-appearing hills of the 1935 crop, in which the disease was so destructive, eight of the lots had no disease in 1936 and the ninth only 2 percent, showing that practically no spread occurred in 1935.

In the course of experimental studies in central Wisconsin from 1933 to 1937, inclusive, tubers from yellow dwarf plants and seed tubers from noninfested areas were planted in adjacent rows in each year except 1936. Seed tubers from the noninfested areas were saved regularly and the disease incidence determined in plantings of the following season. The results of this study in table 7 show that the natural spread was light in 1933, 1934, and 1935 as compared with the extensive dissemination that occurred in 1937.

TABLE 7.—*The natural spread of yellow dwarf in central Wisconsin in 1933, 1934, 1935, and 1937, as shown by tests during each succeeding season of tubers from healthy stock grown alongside yellow dwarf plants*

Year of exposure	Year of test	Hills tested	Nonemerging hills	Hills with yellow dwarf
		Number	Percent	Percent
1933.....	1934	300	5	2
1934.....	1935	200	1	9
1935.....	1936	300	1	6
1937.....	1938	760	28	28

The comparative spread of the virus in various localities in the State in a given year was studied in 1934 and again in 1937. In the former year adjacent rows of yellow dwarf tubers of Russet Rural

variety and tubers of a yellow dwarf-free stock of Rural New Yorker were planted near Ashland in the extreme northern part of the State, at Almond in the central area, and at Madison in southern Wisconsin. The relative behavior of the yellow dwarf tubers has already been discussed and the data are presented in table 4. The best stand was in the northern location and thus a greater abundance of inoculum for insect spread was available there than at the other two locations. From each of the exposed healthy hills tubers were saved from which plantings were made in central Wisconsin in 1935 to determine the relative spread at the three locations. The results are given in table 8. They show that spread occurred in all three areas but was least in the northern location.

TABLE 8.—*The relative spread of yellow dwarf in northern, central, and southern Wisconsin in 1934 as shown by planting tests in 1935 of tubers from hills exposed in those areas*

Location in which plants were exposed in 1934	Relative location in Wisconsin	Hills planted in 1935	Hills showing yellow dwarf in 1935 tests
		Number	Percent
Ashland.....	Northern.....	200	1
Almond.....	Central.....	200	9
Madison.....	Southern.....	300	7

In 1937 a series of potato plots set up by the departments of horticulture and genetics, University of Wisconsin, in which 19 varieties or strains were grown in 4 randomized replicate blocks in 7 different locations in the State. Four of these were in northern locations—Barron, Oneida, Langlade, and Door Counties. One was at Almond, Portage County, in central Wisconsin. Two were in the southeastern portion of the State—Washington and Walworth Counties. In one of the strains a small percentage of yellow dwarf infection occurred in the seed stock, and thus there were a few secondarily infected plants at each location. In the Almond location the plot adjoined a large field of Russet Rural potatoes in which about 3 percent of the hills showed secondary yellow dwarf infection from the seed pieces. An occasional yellow dwarf plant occurred in Washington and Door Counties in the fields of which the variety plots were each a part. In the remaining locations the only yellow dwarf observed was that introduced in the single strain already noted.

The spread during 1937 was very pronounced at Almond. By September 1 primary symptoms were to be found on most plants in the field. There was a very large population of the clover leafhopper (*Aceratagallia sanguinolenta* (Prov.)) in this section. While the insect was found in the other southern locations it was not abundant and it was absent or rare in the northern counties.

A random sample taken from each composite of the four replicates of each variety at each location was planted in 1938 at Hancock in central Wisconsin. In the case of stocks from Portage and Washington Counties 40 tubers were saved from each and planted at the rate of 1 tuber per hill. From each of the other locations 20 tubers were used, each tuber being divided between 2 hills. The occurrence of yellow dwarf resulting from tuber infection was recorded and the results are presented in table 9.

TABLE 9.—The relative spread of yellow dwarf in 19 strains of potato grown in randomized replicate blocks in 7 locations in Wisconsin in 1937¹

Variety or strain	Percentage of missing hills (mh) and yellow dwarf (yd) in tests ¹ at Hancock in central Wisconsin in 1938 from stocks produced in 1937 in—													
	Barron County		Oneida County		Langlade County		Door County		Portage County		Washington County		Walworth County	
	mh	yd	mh	yd	mh	yd	mh	yd	mh	yd	mh	yd	mh	yd
Triumph.....	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
Warba.....	5	0	3	0	8	0	5	0	53	28	3	0	0	0
Irish Cobbler.....	0	0	10	0	3	0	5	0	5	13	0	0	0	0
Cottrell Cobbler.....	5	0	0	0	0	0	8	0	18	28	3	0	0	0
100-Day Cobbler.....	5	0	0	0	0	0	0	0	25	45	0	0	0	0
White-blossomed Cobbler.....	0	0	0	0	0	0	3	0	15	18	0	0	0	0
Earlaine.....	5	0	5	0	0	0	18	0	68	5	0	0	0	0
Mesaba.....	0	0	10	0	0	0			38	23	5	0	0	0
Houma.....	15	0	8	0	0	0	0	0	30	40	0	0	0	0
Rural New Yorker.....	8	0	3	0	0	0	0	0	15	38	0	0	3	0
Pioneer Rural.....	0	0	0	0	0	0	3	0	38	13	0	0	3	0
Toanaco 4.....	0	0	3	0	0	0	0	0	20	30	0	0	5	0
Russet Rural.....	15	0	15	0	0	0	5	0	48	23	3	0	0	0
Russet Rural (Martin strain).....	5	0	0	0	0	0	8	0	45	15	0	0	0	0
Chippewa.....	8	0	3	0	0	0	10	0	43	33	0	0	0	0
Katahdin.....	3	0	3	0	0	0	5	0	8	40	0	0	5	0
Green Mountain.....			3	0	8	0	13	0	13	40	0	0	5	0
Russet Burbank.....	5	0	0	0	0	0	0	0	23	47	3	0	0	0
Columbia Russet.....	0	0	0	0	0	0			5	13	0	0	0	0
Average.....	0	0	0	0	0	0	18	0	13	38	0	0	0	0
	4	0	3	0	1	0	6	0	28	28	1	0	1	0

¹ 40-tuber units of the stocks from Portage and Washington Counties; 20-tuber units were planted from the remaining locations.

Several insects have been reported as vectors of the yellow dwarf virus. Koch (4) claimed transmission by the peach aphid (*Myzus persicae* (Sulz.)). Muncie (7) reported transmission by means of the potato aphid (*Macrosiphum solanifolii* Ashm.) and the potato leafhopper (*Empoasca fabae* (Harris)). Black (2), however, was unable to show any one of these insects to be a vector, but he proved definitely that the clover leafhopper did transmit the virus. He considered the last-named insect to be the chief, if not the only, vector. It is not the intent to report in this paper on the insect-transmission studies that the writers have under way, but it is of interest, in view of the confusion which now exists on this point in the literature, to point out the interesting circumstantial evidence that is presented in the studies reported in table 9. Although yellow-dwarf plants were present uniformly in all locations as a result of the random arrangement, general spread of the virus occurred only in the central-Wisconsin plot. Since the potato leafhopper was quite as prevalent in the Walworth County and Washington County plots as in the central-Wisconsin plot, the lack of yellow dwarf spread in these two locations rules out that insect as an important vector of yellow dwarf in this case. In the Walworth County plot there was severe spread of mosaic, indicating the presence of the potato or peach aphid or both in considerable numbers in that location. Since no spread of yellow dwarf occurred, aphids would seem not to have functioned as important vectors of that disease. At the Barron County plot a general spread of spindle tuber occurred without any spread of yellow dwarf and

only a very limited spread of mosaic. This plot was subjected to a heavier grasshopper infestation than any other of the seven and that insect may well have caused the unusual amount of dissemination of the spindle-tuber virus. These facts, together with the heavy infestation of clover leafhoppers at Almond in 1937, seem to leave little doubt that the latter was the principal vector of the yellow dwarf virus in Wisconsin in that year.

The range of yellow dwarf as a destructive disease in Portage County in the epidemics of 1932 and 1937 was restricted to the

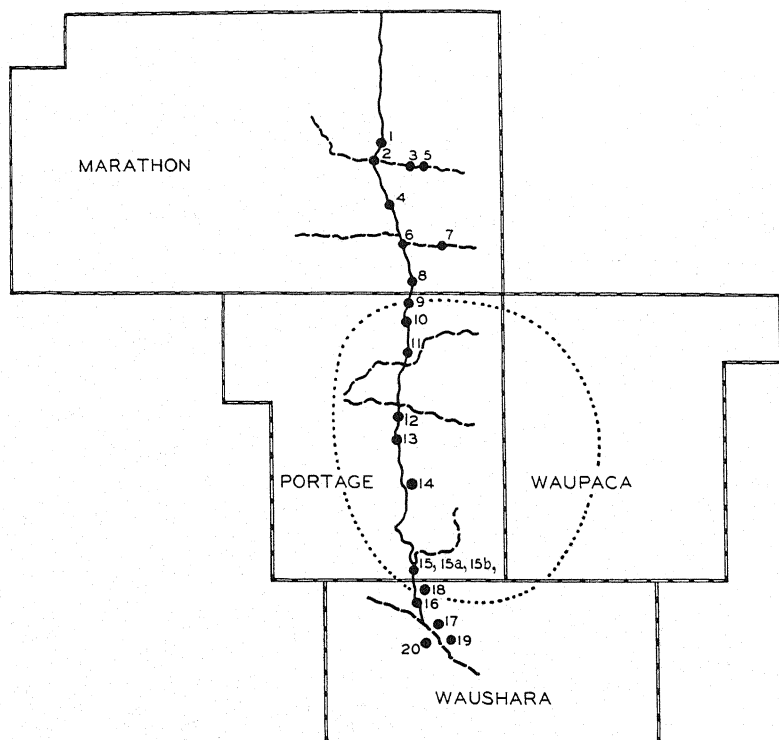


FIGURE 6.—Map of the central Wisconsin area showing location (by number) of farms from which tuber samples were secured in the fall of 1937 for studying the natural occurrence of potato yellow dwarf. The area surrounded by the dotted line is that in which the disease was most severe in 1932 and 1937.

eastern half of the county. The outer fringe of this area runs roughly as indicated in figure 6. In the zone immediately to the north and west of this line many growers have used the same seed stock for a long period of years without any tendency for the yellow dwarf virus to be built up in it, although the virus is not entirely absent, for an occasional diseased plant is found. The samples which were collected at random in 1932 from fields in the area to the north, west, and south of the high-disease region were included in the 1933 trials and comprise those under group 2 in table 1. They showed no yellow dwarf. When the epidemic of 1937 got under way another survey of central Wisconsin showed practically the same relation of low-disease and

high-disease zones as in 1932. Many fields which were a total loss in the high-disease zone were from certified seed stock grown only one season in the locality, while in the low-disease zone many growers had used the same seed stock for 10 to 20 years, i. e., throughout the epidemic of 1932 and the years immediately preceding it.

In order to get further data as to the relative spread in these zones during the 1937 epidemic, samples were collected at harvest from fields at random along the highway indicated in figure 6, extending from the low-disease area on the north through the high-disease area and into the area where the disease had been less severe on the south. These samples were planted in 1938 and the stand and yellow dwarf counts were taken. The results are given in table 10. They show that the concentration of the virus was again greatest in the same zones as in 1932. In 1937 the area to the north remained quite free from general spread of the virus as in previous years. There appeared to be an extension of the severe-disease area to the south since samples collected on the southern fringe (lot Nos. 17-20) showed relatively high percentages of yellow dwarf and nonemerging hills.

TABLE 10.—The occurrence of yellow dwarf and missing hills in plantings made from seed tubers collected in the fall of 1937 from fields at random in the low-disease and high-disease areas of Marathon, Portage, and Waushara Counties, Wis., as indicated in figure 6

Lot No. ¹	Variety	Years of continuous use of seed stock	Non-emerging hills	Yellow dwarf	Lot No. ¹	Variety	Years of continuous use of seed stock	Non-emerging hills	Yellow dwarf
		Number	Percent	Percent			Number	Percent	Percent
1.....	Russet Rural.....	4	3	0	12....	Rural New Yorker.....	3	43	18
2.....	do.....	4	10	0	13....	do.....	3	33	28
3.....	do.....	8	3	0	14....	do.....	2	73	18
4.....	do.....	6	5	0	15....	do.....	2	48	33
5.....	do.....	15	8	0	15a..	Chippewa.....	2	13	60
6.....	do.....	4	8	0	15b..	Spaulding Rose.....	2	28	55
7.....	do.....	7	5	0	16....	Russet Rural.....	20	18	25
8.....	Rural New Yorker.....	7	15	3	17....	do.....	20	13	15
9.....	do.....	10	43	0	18....	do.....	2	13	50
10....	do.....	4	18	13	19....	do.....	15	10	10
11....	do.....	7	68	3	20....	do.....	2	43	13

¹ See fig. 6.

A field survey of clover leafhoppers in 1937 showed them to be quite scarce in the low-disease zone to the north and west and very abundant in the high-disease zone. This, no doubt, is a fundamental reason for the existence of clear-cut limits to the severe-disease area. However, there is no such relation here between the occurrence of red clover (*Trifolium pratense* L.) and the occurrence of the leafhopper and the virus as is claimed by Black (2) and Mader (5) in New York State. In fact, quite the reverse is true, since the high-disease zone in Portage County, being of light acid sandy soil, is a poor clover area and very little of it is grown there. On the other hand, the heavier soil in the low-disease zone just to the north of this county, where the virus and clover leafhoppers are both rare, is a very good red clover area and supports large acreages of it annually. The same may be said of several of the other locations where the plots which were carried in

1937 showed little or no yellow dwarf spread. On the basis of these experiments and observations there is no reason to look upon red clover as the important overwintering host of yellow dwarf in Wisconsin. In the districts where epidemics have been most destructive in this State there must be sufficient carry-over in the insects and in potato tubers or in overwintering hosts other than red clover to provide inoculum in the spring.

DISCUSSION

The object of this investigation has been to throw more light on the development of yellow dwarf under Wisconsin conditions. The disease is distinct from other virus diseases of the potato in that one of the outstanding features is the reduction in emergence of shoots from infected seed tubers. In view of the fact that this vulnerable effect upon the plant is enhanced with increase in soil temperature, it is not surprising that the "poor stand" phase of the disease has been the outstanding one in central Wisconsin. For various reasons which need not be discussed here the majority of the crop is planted relatively late, i. e., during the first half of June. This and the fact that the sandy soil which prevails in that section has a rather high specific heat combine to bring about in many seasons the optimum conditions for severe disease manifestations.

In view of the fact that plants which are infected from the seed piece usually produce tubers that are reduced in size and few in number, the virus might well be largely self-eliminating were it not for its dissemination during the growing season. The environmental studies show that high temperatures of air and soil both tend to enhance the expression of top symptoms while cool air and even cool soil tend to suppress them. Thus it becomes evident that such spread may be general without causing current-season symptoms on tops or tubers depending on the prevailing climate. In a cool season the evidence of current-season spread may be completely masked even though it occurred fairly early in the season. The uncertainty of maintaining yellow dwarf-free seed stocks in some areas is therefore greatly increased.

The report of Black (2) that the clover leafhopper is a vector of the virus has been confirmed. Studies of the epidemic of 1937 in central Wisconsin show a close correlation of seasonal spread of the virus with the abundance of that insect. A comparison of comparable seed lots grown in various parts of the State shows that there is no correlation of yellow dwarf spread with the occurrence of the potato leafhopper, nor with the dissemination of aphid-transmitted viruses such as those of the mosaics and spindle tuber.

It should also be made clear that the section in which yellow dwarf has been most serious in Wisconsin supports a negligible acreage of red clover. This crop does not appear to be an important source of overwintering inoculum. On the contrary, an area only a few miles removed from that in which yellow dwarf occurs to some extent annually and in severe epidemic form periodically has remained free from more than a negligible occurrence of the disease for the past 7 years or longer in spite of the fact that it supports a substantial acreage of that legume. The main source of overwintering inoculum in the yellow dwarf section of central Wisconsin may well be the seed tuber, while the extent of seasonal spread and subsequent development of

secondary symptoms in epidemic form may depend largely upon the relative occurrence of clover leafhoppers.

In the 1937 epidemic in Portage County, 19 varieties or strains of potato were exposed to natural infection. While the 1938 tests (table 9) show that each of these clones produced some infected tubers in 1937, it should be pointed out that Russet Burbank was distinctly the lowest of the group when percentages of both missing hills and yellow dwarf are considered. This is in accord with the observation of Taylor (8) who found this variety to be the least infected in a group exposed to natural infection in New York State. Inasmuch as certain individuals of the clone became infected it would seem plausible that the noninfected ones were escapes. The possibility of escape because of a preferred position as to exposure in 1937 is very remote, since the sample of seed tubers was taken from a mixture of the yield of a replicate from each of four blocks in which the arrangement of varieties was randomized. Even though this may prove to be a case of escape, dependent presumably upon the avoidance of the variety by the insect vector, its occurrence in both New York and Wisconsin is sufficiently significant to warrant further study.

SUMMARY

The symptoms of yellow dwarf under Wisconsin conditions are discussed. One important phase of the symptomatology not previously reported is the nonemergence of plants from infected seed tubers.

The study of temperature relations shows that the top symptoms develop most rapidly and severely at high air temperatures and they may be entirely suppressed at 16° C. Low soil temperatures favor germination and emergence from infected seed tubers and tend to suppress the appearance of top symptoms. High soil temperatures tend to prevent emergence and to hasten the appearance of top symptoms. The "poor-stand" phase of yellow dwarf in the field is associated with high soil temperatures in the period.

The sporadic appearance of yellow dwarf in epidemic form is discussed. It is shown that the greatest amount of dissemination in 1937 was in the eastern part of Portage County in central Wisconsin where the clover leaf hopper was most prevalent. No field evidence of spread by the potato leafhopper or aphids was secured.

A study of contiguous low-disease and high-disease areas in central Wisconsin during the period from 1932 to 1938 shows no correlation between red clover plantings and yellow dwarf epidemics. It appears that other sources of inoculum are more important in central Wisconsin.

It is pointed out that Russet Burbank tended to escape infection in the section in central Wisconsin where 18 other varieties or strains of potato became heavily infected in the epidemic of 1937.

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EFFECT OF RELATIVE HUMIDITY ON VIABILITY, MOISTURE CONTENT, AND RESPIRATION OF WHEAT, OATS, AND BARLEY SEED IN STORAGE¹

By D. W. ROBERTSON, *agronomist*, A. M. LUTE, *seed analyst*, and ROBERT GARDNER, *associate agronomist*, Colorado Agricultural Experiment Station

INTRODUCTION

It is well known that seeds deteriorate more rapidly in humid than in arid climates. Under dry conditions some agricultural seeds may be stored for 10 years or more without sufficient loss in viability to make their use unprofitable. In a previous study, Robertson and Lute² found that wheat, oats, and barley stored in a dry room for 15 years retained over 80 percent of their original ability to germinate. In a humid atmosphere, these seeds may lose as much in a few months.

In humid regions the detrimental effect of moisture presents a problem of economic importance when seeds are stored for long periods. The first step in solving this problem is, obviously, a study of the tolerance of seeds to atmospheric moisture. If it were known how much moisture the seeds would tolerate for a given length of time at a given temperature, this information could be used as a guide in selecting storage conditions.

In an attempt to solve this problem an experiment was set up in 1932 in which the effect of atmospheric humidity on rate of loss in viability, respiration rate, and moisture content of wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) was studied.

Respiration rate was studied primarily to see how closely respiration was related to loss in viability. Moisture content was determined because this variable is a function of the humidity and may be used as an index of humidity where no direct measurements are available.

MATERIALS AND METHODS

Humidity control was obtained by the use of glass desiccators containing sulfuric acid solutions. The moisture content of the grain in storage was determined by weighing each sample used for germination as it went into and came out of the desiccators. Percent moisture was calculated on the oven-dry basis. The moisture percentage was considered as the total difference. This, undoubtedly, caused a slight error since loss of solids through the process of respiration was considered as moisture. This error is noticeable in table 6, which shows the moisture content of the grain when the various germination samples were taken. However, the discrepancy is slight when compared with the total amount of moisture contained in the grain at the end of the test period. Respiration rate was estimated on the basis of carbon dioxide found in samples of the air in the desiccators.

¹ Received for publication January 17, 1939.

² ROBERTSON, D. W., and LUTE, A. M. GERMINATION OF SEED OF FARM CROPS IN COLORADO AFTER STORAGE FOR VARIOUS PERIODS OF YEARS. Jour. Amer. Soc. Agron. 29: 822-834. 1937.

Marquis wheat, Colseess barley, and Colorado 37 oats grown in 1922, 1926, and 1931 were used in the experiment. The samples were taken from the same lots studied by Robertson and Lute.²

Fifteen crystallizing dishes, each containing a sample of 200 seeds, were placed in each desiccator over sulfuric acid solutions. Each desiccator had a capacity of 8,400 cc., and the crystallizing dishes were 50 mm. in diameter by 35 mm. deep. The acid varied in concentration in the different desiccators to produce the range in humidity. The data of Wilson³ were used in calculating the concentrations of acid required.

The experiment was run in a thermostatically controlled room at approximately 70° F., except that the thermostat was disconnected while the last set of samples was still under study and these were subjected to a wide range of temperature for the last year, the temperature often reaching 100°.

At suitable intervals, samples of from 500 to 1,000 cc. of air were drawn from the desiccators and analyzed for CO₂. At the same time grain samples were removed for moisture and germination determinations.

The absorption apparatus described by Gardner⁴ was used for the CO₂ analyses. The samples of gas were drawn through 25 cc. of 0.1 normal NaOH in the absorbing flask which contained also a few drops of normal butyl alcohol and phenolphthalein. After the samples were drawn, the flask was disconnected and 10 cc. of 10-percent BaCl₂ added. The contents were then titrated with 0.1 normal HCl in the closed flask through a hole in the stopper.

After each sampling, the lids of the desiccators were removed and the air changed by fanning. The CO₂ in the outside air was determined at each change of air and a correction made for this at the time of the next sampling. A blank determination was used to correct for the CO₂ initially in the absorption flask.

The experiment was run, first with seed treated with Ceresan, but later the possibilities of using copper carbonate, mercuric chloride, and formaldehyde were studied, and finally untreated seed was used.

EXPERIMENTAL RESULTS

The use of an excess of Ceresan where a gram or more of this material was shaken with the seed and the excess not removed was found to be injurious at high humidities. This effect is shown in tables 1 and 2. In table 1 the first and third sets of seed were treated with an excess of Ceresan. Percent germination dropped very low within a week in nearly every case. The second set treated with an excess of copper carbonate deteriorated more slowly. Table 2 shows that seed in a saturated atmosphere, covered with Ceresan, was killed within 7 days, while seed untreated or lightly dusted with Ceresan survived much longer.

Table 3 shows the moisture absorbed, the rate of CO₂ production, and the percent germination of 1931 wheat in a saturated atmosphere for a period of 28.5 days measured at 1- to 2-day intervals. In this test, grain was treated with an excess of Ceresan and then shaken

² WILSON, ROBERT E. HUMIDITY CONTROL BY MEANS OF SULFURIC ACID SOLUTIONS, WITH CRITICAL COMPILATION OF VAPOR PRESSURE DATA. Jour. Indus. and Engin. Chem. 13: 326-331, illus. 1921.

⁴ GARDNER, ROBERT. A CONVENIENT ABSORPTION AND TITRATION FLASK FOR CARBON DIOXIDE DETERMINATION. Indus. and Engin. Chem., Analyt. Ed. 7: 437-438. 1935.

over a sieve to remove the excess. The data show that viability decreased more slowly than when the excess Ceresan was not removed, but quite irregularly, indicating that some lots were injured by the treatment.

TABLE 1.—*Viability and moisture content of wheat, oats, and barley when treated with an excess of Ceresan and copper carbonate, and stored in air at 100 percent relative humidity for periods up to 85 days in preliminary tests made at Fort Collins, Colo.*

Grain and time in desiccator (days)	Germination of—			Moisture content of—		
	Lot 1 ¹	Lot 2 ²	Lot 3 ¹	Lot 1 ¹	Lot 2 ²	Lot 3 ¹
	Percent	Percent	Percent	Percent	Percent	Percent
Wheat:						
0.....	95.5	95.5	95.5	5.09	5.72	5.72
7.....		97.0	46.0		17.62	21.78
14.....		50.0	21.0		20.87	24.35
21.....		49.0	0		23.52	27.92
28.....		29.0	0		23.71	30.20
35.....		6.0	4.0		26.89	29.72
42.....		0	5.0		32.60	29.96
49.....		0	44.0		32.55	
70.....	16.0					
85.....				38.95		
Oats:						
0.....	97.0	98.5	98.5		6.16	6.16
7.....		99.0	86.0		19.43	21.65
14.....		59.5	2.0		22.49	24.28
21.....		51.0	0		24.65	26.70
28.....		39.0	0		26.62	29.68
35.....		5.0	0		29.63	30.64
42.....		0	0		31.79	27.68
49.....		0	0		33.84	
70.....	0					
85.....	0					
Barley:						
0.....		96.5	96.5		5.43	5.43
7.....		98.0	6.0		19.43	20.08
14.....		89.5	69.0		22.80	23.38
21.....		68.5	2.0		24.63	26.31
28.....		68.0	14.0		26.33	29.31
35.....		35.0	0		28.87	31.45
42.....		0	1.0		19.95	29.33
49.....		0	1.0		30.56	
70.....	21.5					
85.....	16.0			24.02		

¹ Treated with an excess of Ceresan; excess not removed.

² Treated with an excess of copper carbonate.

TABLE 2.—*The effect of Ceresan treatment (excess not removed) on the viability of Marquis wheat stored for periods up to 28 days at 100 percent relative humidity*

Time (days)	Germination			Time (days)	Germination		
	No treatment	Dusted	Covered		No treatment	Dusted	Covered
	Percent	Percent	Percent		Percent	Percent	Percent
7.....	93.5	90.0	0	21.....	46.5	65.0	
14.....	88.5	54.0	0	28.....	38.5	36.0	

Table 4 shows the viability of wheat, oats, and barley for 527 days at humidities ranging from 70.2 to 6.7-percent saturation. In the extremely dry atmospheres very little injury was noted during the period, though all seed received the excess Ceresan treatment. At 42.3-percent saturation (table 4), the percent germination for wheat was slightly lower at the end of 527 days than the untreated grain after 1,032 days (table 5), indicating some damage at this humidity.

TABLE 3.—Effect of Ceresan treatment on the moisture absorption, the CO₂ production, and the viability of Marquis wheat (1931 crop) when stored at 100 percent relative humidity and 86° F. for periods up to 28.5 days

[Grain treated with an excess of Ceresan then shaken over a sieve to remove the excess]

Time in desiccator (days)	Moisture absorbed	CO ₂ produced per 1,000 gm. of grain per day	Germination	Time in desiccator (days)	Moisture absorbed	CO ₂ produced per 1,000 gm. of grain per day	Germination
	Percent	Milligrams	Percent		Percent	Milligrams	Percent
0.0.....	9.1	98.5	13.5.....	35.6	121.5	65.0
1.5.....	17.0	1.9	95.0	14.5.....	36.3	138.3	70.0
2.5.....	21.9	5.1	94.5	15.5.....	37.3	151.6	81.0
3.5.....	23.2	17.2	91.0	16.5.....	36.6	97.5	85.0
4.5.....	27.6	12.7	81.0	17.5.....	37.1	150.8	72.0
5.5.....	28.4	29.5	84.5	18.5.....	37.4	99.6	80.5
6.5.....	29.3	50.1	75.0	19.5.....	39.7	210.9
7.5.....	32.3	58.5	84.5	20.5.....	39.7	172.9	70.5
8.5.....	32.3	57.7	91.5	22.5.....	41.3	119.6	80.0
9.5.....	34.4	64.7	80.0	24.5.....	37.7	149.5	66.5
10.5.....	35.1	85.1	82.5	26.5.....	37.7	130.2	59.5
11.5.....	35.7	96.1	87.5	27.5.....	39.4	106.7	38.0
12.5.....	36.6	116.5	86.5	28.5.....	39.7	81.1	89.0

The reduction in germination was slightly less for oats and barley than for wheat. Particularly this is true for the grain stored at 42.3-percent humidity. A possible explanation of this difference may be found in the protection afforded the germ by the hulls of oats and barley.

TABLE 4.—Viability of wheat, oats, and barley produced in 1922, 1926, and 1931, when treated with an excess of Ceresan and stored in air at different relative humidities for periods up to 527 days

Grain and time in desiccator (days)	Germination at—											
	70.2 percent humidity			42.3 percent humidity			19.1 percent humidity			6.7 percent humidity		
	1922 seed	1926 seed	1931 seed	1922 seed	1926 seed	1931 seed	1922 seed	1926 seed	1931 seed	1922 seed	1926 seed	1931 seed
Wheat:	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
0.....	93.5	92.0	95.5	93.5	92.0	95.5	93.5	92.0	95.5	93.5	92.0	95.5
71.....	36.0	50.3	63.5	74.5	83.0	85.0	90.5	90.0	90.5	93.5	89.0	84.5
114.....	28.0	70.0	81.5	88.5
142.....	21.5	73.0	85.0	93.5
170.....	31.0	57.0	67.5	63.0	81.5	86.0	86.5	83.5
302.....	.0	1.0	27.0	52.0	56.0	51.0	82.0	75.0	67.0	86.5	85.5	85.0
527.....	.0	.0	.0	47.0	52.5	48.0	80.0	81.0	67.5	80.0	77.5	73.0
Oats:	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
0.....	92.5	97.0	97.0	92.5	97.0	97.0	92.5	97.0	97.0	92.5	97.0	97.0
71.....	25.5	64.0	23.5	75.0	92.5	86.5	87.5	98.5	89.0	87.0	95.5	100.0
114.....	51.5	83.0	86.5	91.0
142.....	57.0	86.5	77.5	86.0
170.....	10.05	89.0	85.5	89.5	87.5	95.0	87.6
302.....	.0	1.0	.0	69.0	89.5	76.0	81.5	92.0	87.0	83.0	88.0	84.0
527.....	.0	.0	.0	78.0	84.0	73.0	82.0	91.0	82.0	82.0	95.0	84.0
Barley:	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
0.....	90.0	95.5	96.5	90.0	95.5	96.5	90.0	95.5	96.5	90.0	95.5	96.5
71.....	84.5	93.5	92.5	90.8	91.5	90.5	90.0	86.5	94.0	94.0	94.0	97.0
114.....	74.0	86.5	91.5	90.0	89.5
142.....	69.5	89.5	89.5	92.0
170.....	75.0	85.0	90.0	86.5	88.0	87.5	94.5
302.....	1.0	31.0	60.0	87.0	86.0	84.0	90.0	91.0	83.0	93.0	93.0	94.0
527.....	.0	.0	.0	79.5	82.5	87.5	87.0	82.0	82.0	90.0	88.0	91.5

Trials were next made with seed treated with HgCl_2 by the method of Norton and Chen⁵ and with a formaldehyde solution (1 pint of 40-percent formaldehyde diluted with 40 gallons of water). The HgCl_2 treatment killed all the seed within 4 days and the formaldehyde treatment was ineffective in preventing mold.

The experiment was then continued with untreated seed over a humidity range of from 98-percent saturation to 57.6-percent saturation. The results are shown in tables 5, 6, and 7, and graphically in figure 1. These results should be comparable to conditions of normal

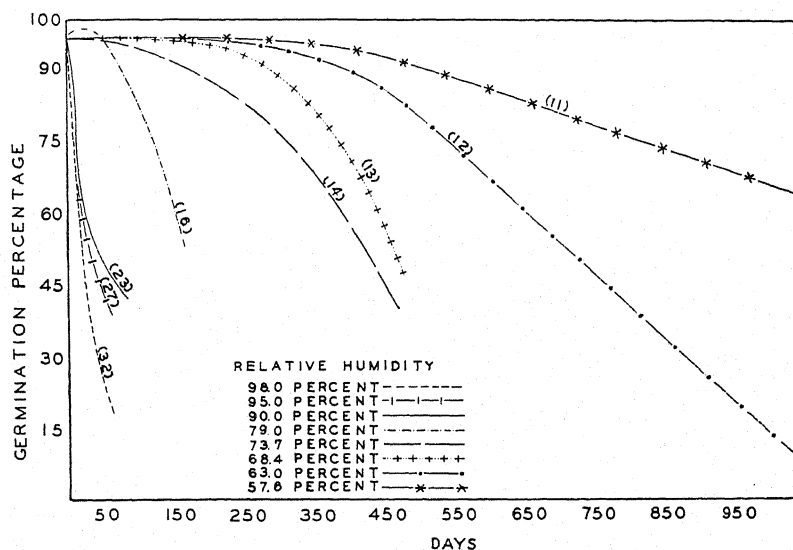


FIGURE 1.—Average viability and moisture content of wheat, oats, and barley grown in 1931 and stored in desiccators at different relative humidities for 1,032 days or less. Each curve represents a different relative humidity. Moisture content of the grain at the end of the storage period is given in parentheses.

storage since stored grain is not ordinarily treated with fungicides. It is possible that fungi as well as normal deterioration accompanying respiration were responsible for part of the loss in viability at the higher humidity. Mold developed on all of the grain at 79-percent saturation or higher. It will be noted from table 5 that the rate of loss in viability is very much accelerated as the humidity increases from 57.6-percent saturation to nearly saturation. Pronounced injury is noted in less than a month at 90 percent or higher. Three samples still germinated quite well at the end of 1,032 days at the lower humidity, though most of the germination dropped to a low figure before that time. The older grain showed the most rapid decline in germination at all humidities.

⁵ NORTON, J. B. S., and CHEN, C. C. SOME METHODS FOR INVESTIGATING INTERNAL SEED INFECTION. *Phytopathology* 10:399-400. 1920.

TABLE 6.—*Moisture content of untreated wheat, oats, and barley grown in 1922, 1926, and 1931, when stored in desiccators in atmospheres of different relative humidities for periods up to 1,032 days*

[illegible]

TABLE 7.—CO₂ produced per 1,000 gm. per day of untreated wheat, oats, and barley when stored in desiccators in atmospheres of different relative humidity for periods up to 1,032 days

Grain and time in desiccator (days)	CO ₂ produced per 1,000 gm. of grain per day at—							
	98 per cent humidity	95 per cent humidity	90 per cent humidity	79 per cent humidity	73.7 per cent humidity	64.8 per cent humidity	63.0 per cent humidity	57.6 per cent humidity
Wheat:	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams	Milli-grams
0-14	36.0	35.7	9.4	2.1				
14-20	113.4	112.1	88.5					
20-30	146.8	78.2	58.1					
14-35				5.2				
30-38	345.7	81.7	34.4					
38-56	293.6	142.7	30.2					
35-100				28.7				
100-158				37.6				
0-158					1.5			
158-219					3.8			
0-181						0.0		
219-300					6.2	0		
0-300							0.0	
300-454					9.6	2.0	0	
0-454								0.1
454-1,032					1.5	2.7	.7	.1
Barley:								
0-14	35.2	27.6	9.4	2.0				
14-20	103.2	90.0	88.5					
20-30	94.3	54.5	58.1					
14-35				3.4				
30-38	178.1	62.3	24.4					
38-56	255.3	57.0	30.2					
35-100				28.4				
100-158				8.5				
0-158					2.0			
158-219					7.8			
0-181						.3		
219-300					3.9	0		
0-300							.1	
300-454					6.6	1.4	.3	
0-454								.4
454-1,032						.1	.5	.3
Oats:								
0-14	46.9	41.0	18.9	2.3				
14-20	148.3	126.9	107.4					
20-30	137.2	86.9	55.1					
14-35				4.1				
30-38	211.6	85.1	44.7					
38-56	279.8	119.3	38.0					
35-100				36.3				
100-158				27.8				
0-158					3.9			
158-219					8.4			
0-181						.4		
219-300					9.5	0		
0-300							.3	
300-454					6.3	4.1	0	
0-454								.4
454-1,032					2.1	5.2	.1	.2

The data in table 6 show the moisture content of grain at each humidity. It will be seen that the initial moisture in the grain before the experiment started was a little more than 2 percent lower than that found at 57.6-percent saturation at the end of 1,032 days of storage. Figure 1, in which the average moisture of the grains grown in 1931 is given on the different humidity curves, shows a decrease from 32 percent to 11 percent as the humidity decreased from 98.0- to 57.6-percent saturation. Table 5 shows that severe injury resulted in less than 1,032 days at 57.6 percent, in less than 454 days at 68.4 percent, in less than 100 days at 79 percent, and in less than 30 days above 90 percent. In figure 1, in which the average germination of wheat, oats, and barley grown in 1931 is plotted against

time, the general trend of germination is clearly shown at the different relative humidities.

The tests indicate that equilibrium is reached in about 30 days for grain stored in atmospheres of over 79.0-percent relative humidity. For humidities lower than this, the exact time required for equilibrium to be reached was not determined. However, the indications are that all of the grains had reached equilibrium by the time the first moisture sample was taken. The data show that stored grain tends to reach equilibrium with the surrounding air in a rather short period. The moisture equilibrium of the different grains varied slightly in atmospheres of the same relative humidity.

Table 7 shows the respiration rate as measured by CO_2 production and indicates a close relationship between respiration, moisture content of grain, and atmospheric moisture. Whether loss in viability is a direct result of respiration or merely incidental to respiration is not deducible from the experiment.

DISCUSSION

While the results of the experiment show that the decrease in viability due to moisture in the atmosphere depends on the age and condition of the grain when it goes into storage as well as on the relative humidity, they also show that grain in even the best condition will not survive long when the atmospheric moisture approaches saturation at the temperature studied. For example, very serious damage occurred in less than a month to all grain stored at 90-percent relative humidity or higher, while some of the grain lasted nearly 3 years with small damage at 57.6 percent (fig. 1). Similar results are reported for oats by Bakke and Noecker.⁶ They found that grain containing 15 percent of moisture and less showed a germination of 91 percent and above at the end of the experiment. Grain containing a greater percentage of moisture ranged from 3 to 85 percent in germination at the end of the experiment. Their experiments were of short duration.

The data in the germination table should be useful as a guide in predicting the approximate maximum time seed can be kept in storage at the temperature studied without serious injury. Since the percentage of moisture in the grain may be used as an estimate of the approximate humidity of the storage environment, it may also be used directly in estimating the probable maximum life of the grain. It will be noted from the moisture tables and figure 1 that approximate equilibrium is reached within 2 weeks when grain is stored in atmospheres above 79-percent relative humidity. On the basis of these figures, grain containing more than 20 percent of moisture cannot be expected to last more than a month without serious injury if the moisture content does not decrease; while grain with 10 percent of moisture might last as long as 3 years if in good condition at the beginning. Either the relative humidity or the percentage of moisture in the grain can be used as a valuable criterion in predicting the probable rate of deterioration of wheat, oats, and barley in storage.

⁶ BAKKE, A. L., and NOECKER, N. L. THE RELATION OF MOISTURE TO RESPIRATION AND HEATING IN STORED OATS. Iowa Agr. Expt. Sta. Research Bull. 165, pp. [319]-336, illus. 1933.

SUMMARY

A study of the effect of relative humidity on the rate of loss in viability of wheat, oats, and barley in storage gave the following results:

(1) Heavy treatments with Ceresan before storage decreased the length of life of seeds stored at high humidities.

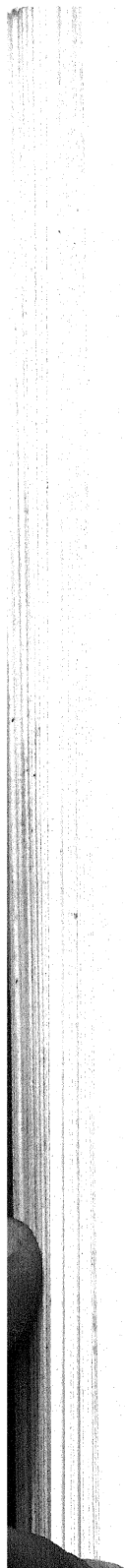
(2) The length of life of both treated and untreated seeds in storage increased as the humidity decreased over the range studied. Serious injury was suffered by all grains at 100-percent saturation within a month. The injury decreased with decreased humidity. Only a slight loss in viability was found in some samples at the end of 1,032 days' storage in an atmosphere of 57.6-percent saturation.

(3) Respiration, as measured by CO_2 production, increased regularly with relative humidity.

(4) Moisture percentage changed more consistently with humidity than either viability or respiration.

(5) The data showing the rates of change of moisture and viability with humidity offer a means of predicting the maximum time which would be safe for storage under any given relative humidity, assuming temperature conditions comparable to those of the experiment.

(6) Heavy fungus growth developed on all grain at the higher humidity when the grain was not treated with a fungicide. The data do not show what influence these organisms and the bacteria that probably accompanied them had on the decrease in viability of the seed.



SIGNIFICANCE OF VARIATION IN HAM CONFORMATION¹

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INTRODUCTION

The conformation of the hams is one of the major characteristics considered in the selection of hogs for slaughter. A relatively short, plump, well-muscled ham having a moderately thick covering of external fat and a high proportion of edible meat is the type in greatest demand by consumers. The objects of the study here reported were to determine the relationships of certain anatomical factors to this desired conformation and to obtain additional facts regarding their scientific and practical significance.

Previous work² showed that as the type of hog ranges from large to small there is an improvement in the conformation or increase in plumpness of the hams. In a later study³ it was found that with these changes there were increases in the percentages of separable fat and total edible portion and decreases in separable lean and skin and bone of the ham. The most striking change was in the proportion of fat.

EXPERIMENTAL PROCEDURE

The hams used in this study were from Poland China hogs produced at the Agricultural Research Center, Beltsville, Md. Of the total of 59 hams, 9 were from large-type hogs, 38 from intermediate, and 12 from small-type hogs. Beginning at 72 days of age, all animals received, in self-feeders, No. 2 yellow shelled corn and a mixture of tankage 50 parts, linseed meal 25 parts, and alfalfa leaf meal 25 parts, with an adequate mineral mixture. As they individually reached the weights of approximately 225 pounds the hogs were slaughtered. The actual range in final feed-lot weight was from 212 to 242 pounds.

Immediately prior to slaughter the animals were judged individually for type by a committee of three qualified judges employed in the United States Department of Agriculture. After the carcasses had been chilled for 72 hours, the index of ham plumpness was determined, based on measurements taken as follows: (1) Obtaining the length from the lowest point of the aitchbone to the center of the inside of the hock joint located at the bony projection that may be felt under the skin; (2) determining the circumference at the midpoint of the first measurement by locating three or four points on the ham equidistant from the plane through the center of the hock joint, such points being marked with sharp metal skewers and the ham being encircled with a steel tape immediately below the skewers for measurement; and (3) multiplying the second measurement by 100 and dividing by the first measurement, thereby obtaining the index of plumpness. Other car-

¹ Received for publication March 3, 1939.

² HANKINS, O. G. RELATION BETWEEN TYPE IN HOGS AND THE PLUMPNESS OF THEIR HAMS. *Amer. Soc. Anim. Prod. Proc.* 27: 120-123. 1934.

³ — and ELLIS, N. R. A STUDY OF HAM COMPOSITION WITH SPECIAL REFERENCE TO TYPE OF HOG. *Amer. Soc. Anim. Prod. Proc.* 28: 111-116. 1935.

cass measurements considered in the study were length of hind leg from the lowest point of the aitchbone to the coronary band of the foot, average thickness of back fat and ham fat, and width of carcass through the hams at the top point of the aitchbone. These measurements and those from which the index of plumpness was derived were taken according to the method reported by Hankins and Ellis.⁴

In removing the hams from the carcass, the cut was made just behind the second sacral vertebra and at right angles to the hind leg. The leg was then cut off through the hock joint. After each ham was trimmed smoothly, the thicknesses of lean and fat at the butt end were measured directly below the pelvic bone.

Each ham was then analyzed physically and the weights and percentages of the separable lean, fat, bone, and skin determined. From

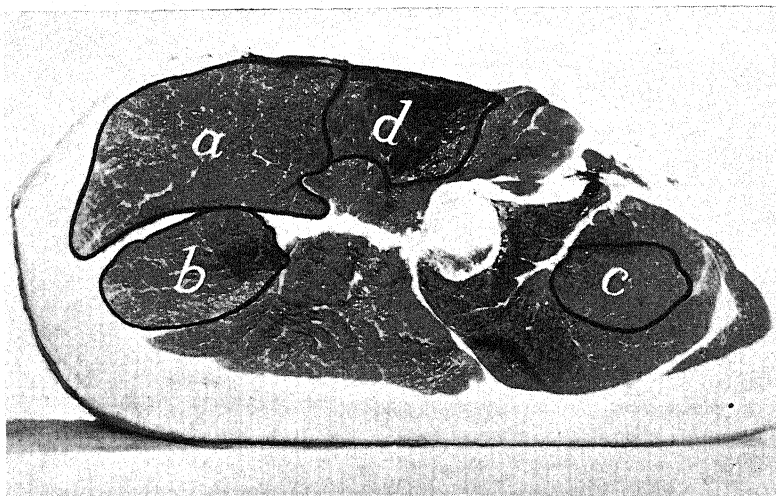


FIGURE 1.—Cross section of ham showing location of muscles studied: *a*, Semimembranosus, *b*, semitendinosus, *c*, rectus femoris, and *d*, adductor.

the separable lean four muscles consisting of the semimembranosus, semitendinosus, rectus femoris, and adductor were dissected out (fig. 1), weighed, and determination made of their percentages based on the total weight of the ham. All components were weighed to the nearest gram.

Because it was the best criterion known, the index of plumpness was used in this study for comparing the hams with respect to conformation. As a first step in the analysis of the data the hams were divided into nine groups, each group having a five-point interval in index of plumpness. This grouping, together with average weights of the hams and various measurements relating to them, is shown in table 1. Figure 2 illustrates hams having the smallest, average, and largest indexes of plumpness of the 59 hams used in the study. The ham with the smallest index is long, narrow, and lacks thickness, whereas the ham with the largest index is relatively short, wide, and thick. The ham of average plumpness is between the two extremes

⁴HANKINS, O. G., and ELLIS, N. R. PHYSICAL CHARACTERISTICS OF HOG CARCASSES AS MEASURES OF FATNESS. Jour. Agr. Research 48: 257-264, illus. 1934.

with respect to these characteristics. Figure 3 shows the relative size and shape of the four muscles taken from the ham with the average index of plumpness, illustrated in figure 2, *B*.

TABLE 1.—*Relationship of index of plumpness of hams to their average weights and measurements*

Hams (number)	Index of plumpness		Weight of ham		Length of ham	Circumference of ham	Thickness of ham fat	Length of ham per kilogram of weight	Thickness of back fat of carcass from which ham was cut	Length of hind leg of carcass from which ham was cut	Width of carcass through ham to length of hind leg, 1 to —	Final feed-lot weight of hogs from which ham was cut
	Range	Average										
			<i>Lbs.</i>	<i>Kilo-grams</i>	<i>Milli-meters</i>	<i>Milli-meters</i>	<i>Milli-meters</i>	<i>Milli-meters</i>	<i>Milli-meters</i>	<i>Milli-meters</i>		<i>Lbs.¹</i>
9.....	120.1-125.0	122.7	15.26	6.895	395.1	484.7	25.9	57.4	37.5	594.7	2.17	225.4
14.....	125.1-130.0	127.6	14.74	6.687	377.9	482.1	26.7	56.7	38.9	587.9	2.05	223.1
11.....	130.1-135.0	132.7	14.81	6.716	373.9	496.0	30.3	55.8	42.5	564.2	2.01	221.9
9.....	135.1-140.0	137.4	14.94	6.776	362.9	498.7	31.7	53.6	42.6	545.1	1.90	222.2
4.....	140.1-145.0	142.8	14.28	6.478	351.5	501.5	28.8	54.3	47.5	535.5	1.87	225.3
2.....	145.1-150.0	146.9	15.12	6.811	347.0	509.5	28.5	51.0	43.7	521.0	1.81	217.5
3.....	150.1-155.0	151.2	14.88	6.751	344.0	520.3	35.3	51.0	50.1	507.7	1.73	221.7
4.....	155.1-160.0	159.2	15.04	6.820	353.3	533.8	35.0	49.3	47.5	497.8	1.69	219.8
3.....	160.1-178.0	169.1	15.12	6.858	325.7	550.7	38.7	47.8	52.0	485.0	1.62	220.7

¹ 1 pound = 0.454 kg.

SIMPLE RELATIONSHIPS OBTAINED

It was found that the weight of the hams did not vary with the index of plumpness of conformation (table 1). However, the length of ham from the lowest point of the aitchbone to the bony projection at the inside of the hock joint showed a consistent decrease with the improvement in conformation. As would be expected, the length-of-leg measurement from the lowest point of the aitchbone to the coronary band of the foot also showed a close but inverse relation to the increase in plumpness. Circumference of ham increased with plumpness, as would also be expected in view of the fact that the index of plumpness represents the relation between circumference and length of the ham.

Both thickness of ham fat and thickness of back fat increased fairly consistently with increasing plumpness of ham, as indicated in table 1. With increasing plumpness there was an uninterrupted narrowing of the relationship between width of carcass through the ham and length of hind leg, and a tendency for a decrease in length from aitchbone to hock joint per unit of weight of ham. In considering the foregoing relationships, it is well to note that the variation in average final feed-lot weights of the hogs was small.

Table 2 shows that the weights of the semimembranosus, semitendinosus, rectus femoris, and adductor muscles did not consistently increase or decrease with increase in ham plumpness. The same statement applies to the percentages of the semitendinosus and adductor muscles, but the proportion of the semimembranosus muscle tended to increase and that of the rectus femoris to decrease with improvement in conformation. The sum of the percentages of the four muscles showed practically no trend associated with increasing plumpness of the hams. In the percentage of total separable lean meat there appeared to be a slight tendency to decrease with increase in

plumpness. On the other hand, the percentage of separable fat and especially the percentage of total edible meat showed a decided tendency to increase with increase in plumpness of the ham.

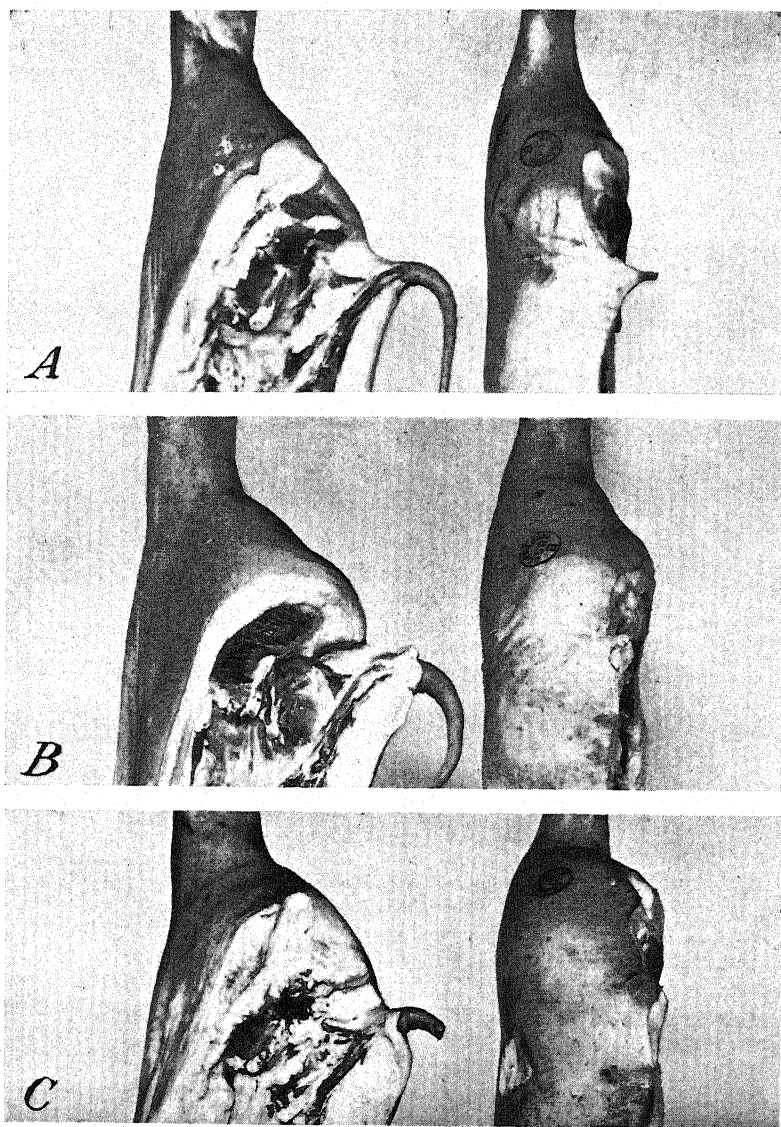


FIGURE 2.—Untrimmed hams having the smallest (A), average (B), and greatest (C) index of plumpness among the 59 hams used in the study. The indexes of plumpness for these three hams were 122.7, 135.5, and 178.0, respectively.

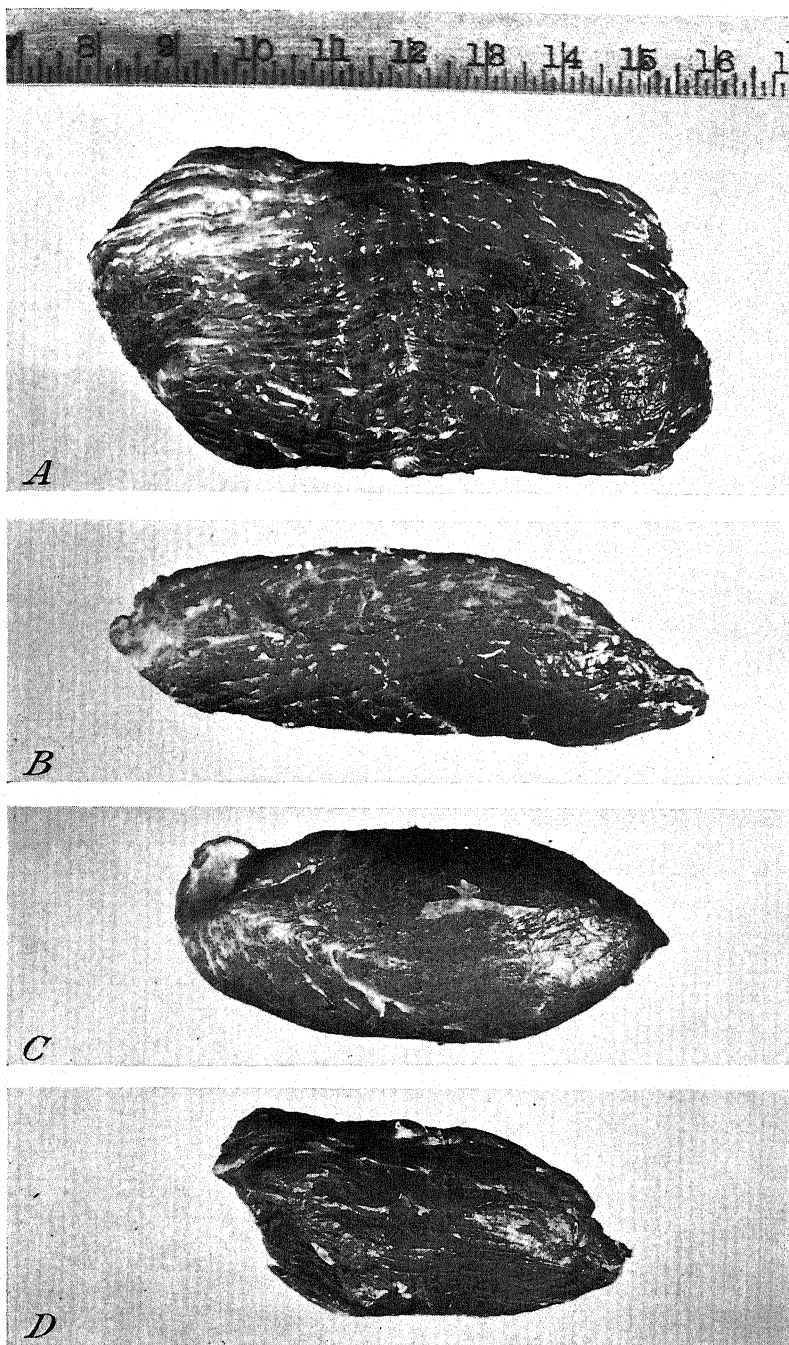


FIGURE 3.—Comparison of size and shape of four muscles taken from ham shown in figure 2, *B*: *A*, Semimembranosus, weight 521.6 gm.; *B*, semitendinosus, 195.0 gm.; *C*, rectus femoris, 249.5 gm.; *D*, adductor, 172.4 gm.

TABLE 2.—*Relationship of index of plumpness of hams to average weights and percentages of the four muscles studied, and percentages of separable fat and lean and total edible meat in the ham*

Hams (num- ber)	Range in index of plumpness	Weight and percentage of muscle									Sepa- rable fat	Sepa- rable lean	Total edible meat
		Semimem- branosus		Semitendi- nosus		Rectus femoris		Adductor		Total			
		Grams	Per- cent	Grams	Per- cent	Grams	Per- cent	Grams	Per- cent				
9.....	120. 1-125. 0	474. 8	6. 85	253. 0	3. 65	264. 1	3. 83	183. 0	2. 65	16. 98	32. 51	53. 00	85. 51
14.....	125. 1-130. 0	485. 7	7. 24	222. 3	3. 32	248. 2	3. 71	174. 3	2. 61	16. 88	34. 84	51. 42	86. 26
11.....	130. 1-135. 0	440. 4	6. 57	204. 1	3. 04	225. 2	3. 36	160. 0	2. 38	15. 35	38. 31	48. 35	86. 66
9.....	135. 1-140. 0	484. 8	7. 15	230. 6	2. 96	227. 8	3. 36	164. 3	2. 42	15. 89	37. 65	49. 69	87. 34
4.....	140. 1-145. 0	491. 0	7. 55	205. 3	3. 16	229. 1	3. 52	175. 8	2. 70	16. 93	35. 87	51. 28	87. 32
2.....	145. 1-150. 0	544. 3	7. 99	224. 5	3. 30	238. 1	3. 50	188. 2	2. 77	17. 56	35. 87	51. 45	87. 15
3.....	150. 1-155. 0	508. 0	7. 53	214. 7	3. 17	219. 2	3. 23	173. 9	2. 58	16. 51	40. 70	47. 82	88. 03
4.....	155. 1-160. 0	511. 5	7. 50	237. 0	3. 50	232. 5	3. 40	166. 7	2. 48	16. 88	39. 39	49. 64	89. 52
3.....	160. 1-178. 0	514. 1	7. 47	231. 3	3. 38	234. 4	3. 41	175. 4	2. 57	16. 83	40. 27	49. 53	89. 80

RELATIONSHIPS SHOWN BY CORRELATION

To obtain further information on the relationships involved in the study, a number of coefficients of correlation with their probable errors were calculated. The values are shown in table 3. According to Fisher,⁵ for *P* of 0.05, with *n* of 59, the correlation coefficient must be at least 0.26, and for *P* of 0.01 the coefficient must be at least 0.33. In table 3 coefficients of 0.26 to 0.32, inclusive, are considered significant and those of 0.33 or more as highly significant.

TABLE 3.—*Coefficients of correlations (with probable errors) of index of plumpness, percentages of separable fat and lean, and weight of ham with indicated factors*

Item	Index of plumpness, <i>r</i>	Percentage of separable fat, <i>r</i>	Percentage of separable lean, <i>r</i>	Weight of ham, <i>r</i>
Percentage of separable fat of ham.....	+0.58±0.06	-----	-0.90±0.02	-----
Percentage of separable lean of ham.....	-.28±.08	-----	-----	-----
Weight of muscle:				
Semimembranosus.....	+ .21± .08	-----	-----	+0.64±0.05
Semitendinosus.....	-.08±.09	-----	-----	+.60±.06
Rectus femoris.....	-.26±.08	-----	-----	+.63±.05
Adductor.....	-.06±.09	-----	-----	+.47±.07
Percentage that muscle is of total ham:				
Semimembranosus.....	+ .27± .08	-0.32±0.08	+.26±.08	-----
Semitendinosus.....	-.10±.09	-.56±.06	+.59±.06	-----
Rectus femoris.....	-.38±.08	-.78±.04	+.75±.04	-----
Adductor.....	+ .02±.09	-.46±.07	+.50±.07	-----
Total four muscles.....	+ .02±.09	-.66±.05	+.75±.04	-----
Thickness of ham fat.....	+ .37±.08	+.79±.03	-.64±.05	-.04±.09
Thickness of back fat.....	+ .77±.04	+.61±.06	-.38±.08	-.13±.09
Ratio of length of hind leg to width of carcass through ham.....	+ .87±.02	-.47±.06	-.28±.08	-----
Ratio of edible to inedible portion in ham.....	+ .79±.03	+.62±.05	-.23±.08	+.32±.08

Table 3 shows that percentages of separable fat and lean, thickness of ham fat, and ratio of edible to inedible portion of ham had highly significant or significant correlations with the index of plumpness. The highest among these values, +0.79, represented the relation between the ratio of edible to inedible portion of ham and plumpness, and the next highest value, +0.58, the relation between percentage of

⁵ FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, 319 pp., illus. Edinburgh and London. 1934.

separable fat and plumpness. As shown by the table, the relation between percentage of separable lean and plumpness was inverse.

Squaring the values mentioned gives 0.62 and 0.34, respectively, as the coefficients of determination for (1) plumpness and percentage of edible meat and (2) plumpness and separable fat. These values indicate that 62 percent of the variance in index of plumpness was associated with a single factor—percentage of edible meat—and that 34 percent of the variance was associated with changes in percentage of separable fat.

Of the four muscles considered, the weight of only one, the rectus femoris, was significantly related to ham plumpness, the coefficient being a negative value (table 3). With respect to percentage, the semimembranosus muscle was significantly related to plumpness and the relationship of the rectus femoris muscle was highly significant but inverse. The percentages of the semitendinosus and adductor muscles had little, if any, relation to the plumpness or conformation of the hams. The same statement applies also to the sum of the percentages of the four muscles.

Both average thickness of back fat and the ratio of length of hind leg to width of carcass through ham had highly significant and relatively close relationships with plumpness of hams. The corresponding coefficients of determination were 0.59 and 0.76.

Since of the several ham characteristics considered the percentage of separable fat was most closely related to plumpness, its relationships with certain other factors were studied. The coefficients representing these relationships are also shown in table 3. Of the correlations between the percentage of separable fat and the percentages of the four muscles, three are highly significant and the fourth, relating to the semimembranosus muscle, is significant. All four coefficients are negative, as would be expected. For the total percentage of the four muscles with percentage of separable fat the correlation coefficient, -0.66 , was highly significant. The corresponding coefficient of determination was 0.44 . Percentage of separable fat was closely related to thickness of ham fat and somewhat less closely related to the ratio of edible to inedible portion of the ham.

The very close inverse relationship between the percentages of separable lean and fat is noteworthy. The relationships of the percentages of the four muscles with percentage of separable lean varied considerably, the coefficients ranging from $+0.26 \pm 0.08$ for semimembranosus, which incidentally was much the largest and heaviest of the four muscles studied, to $+0.75 \pm 0.04$ for rectus femoris. Attention is directed especially to the fact that percentage of separable lean was not a good index of the ratio of edible to inedible portion of ham, although the correlation coefficient was close to the lower limit of the range of significance.

As shown in table 3, the correlations between the weights of the four muscles and weight of ham were highly significant. In none of the four instances, however, was r particularly high. Little relationship was found between thickness of ham fat or thickness of back fat and ham weight. Of greater importance is the fact that ham weight proved to be of little value as an indicator of the proportion of edible meat. The much greater value of the plumpness index for this purpose should not be overlooked.

As already stated, length of ham from aitchbone to hock joint per unit of weight decreased as the index of plumpness increased. A scatter diagram (fig. 4) suggested a curvilinear relationship, and by using a simple parabola the correlation was found to be $\rho=0.82$. The coefficient of determination in this instance was 0.67.

As an additional step, several coefficients of multiple correlation were determined, each representing the relation of index of plumpness,

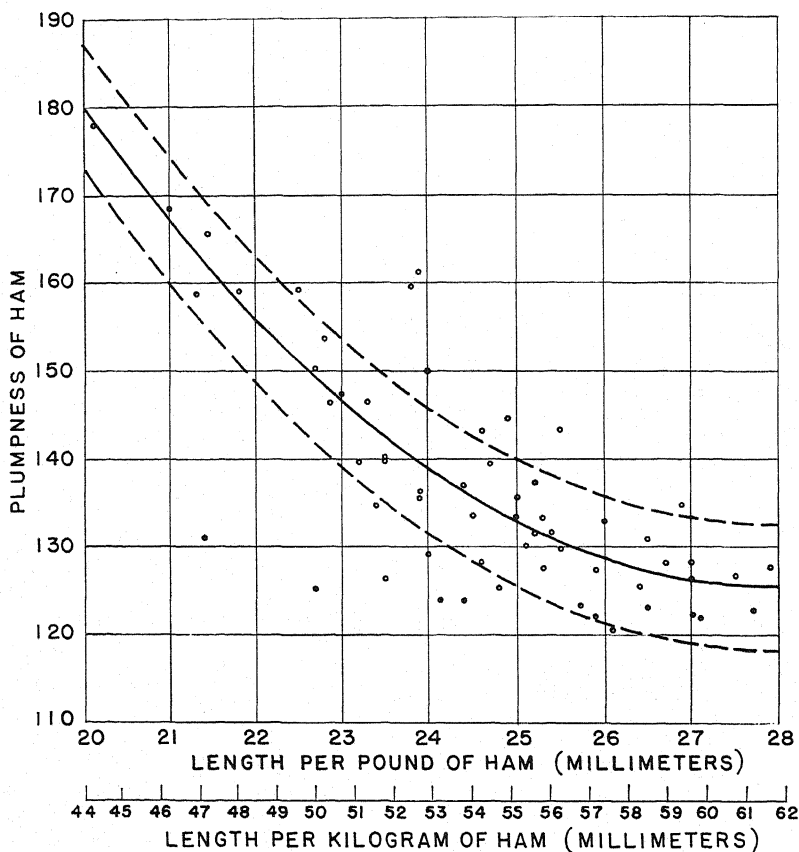


FIGURE 4.—Scatter diagram of index of plumpness and units of length per unit of weight of 59 hams, with curve of average relationship and zones of estimate.

as the dependent variable, to two other ham characteristics. The reason for so doing was the belief that variations in ham conformation might be due more to combinations of factors than to single factors, such as percentage of separable fat and percentage of semimembranosus muscle. As already shown, the simple correlation between index of plumpness and percentage of semimembranosus muscle was $+0.27 \pm 0.08$ and that between index of plumpness and percentage of rectus femoris muscle -0.38 ± 0.08 . The coefficient of multiple correlation, representing the relationship among these three factors, was found to be 0.55. Among the percentages of semimembranosus muscle and separable lean and the index of plumpness the coefficient

of multiple correlation was 0.50. Higher values, 0.62 and 0.80, respectively, were found for the correlations among percentages of separable fat and semimembranosus muscle and index of plumpness and among percentages of separable fat, total percentages for the four muscles, and index of plumpness.

As shown in table 4, the weights of the four muscles were correlated with one another. All the coefficients were positive and highly significant. The semitendinosus and rectus femoris muscles were most closely related, and the semitendinosus and adductor muscles least closely related.

TABLE 4.—Correlations (with probable errors) between weights and between percentages of four muscles in 59 hams varying widely in conformation

Item	Weight of—		
	Semimembranosus muscle	Semitendinosus muscle	Rectus femoris muscle
Weight of—			
Semitendinosus muscle	0.54±0.06	-----	-----
Rectus femoris muscle65±.05	0.73±0.04	-----
Adductor muscle66±.05	.51±.07	0.66±0.05
Item	Percentage in total ham of—		
	Semimembranosus muscle	Semitendinosus muscle	Rectus femoris muscle
Percentage, in total ham, of—			
Semitendinosus muscle	0.30±0.08	-----	-----
Rectus femoris muscle40±.07	0.56±0.06	-----
Adductor muscle60±.06	.74±.04	0.70±0.05

In the percentages of the several muscles, all correlation values, with one exception, were highly significant and all were positive. In the one instance, involving the relation between the semimembranosus and semitendinosus muscles, the coefficient was significant. Of the six relationships represented, the correlation between the semitendinosus and adductor muscles was highest. In general, the adductor muscle was most closely related to the other three. In all cases except the relationships between the semitendinosus and adductor muscles and between the rectus femoris and adductor, the correlation values for weights were higher than for percentages.

SUMMARY AND CONCLUSIONS

Study was made of 59 hams from Poland China hogs slaughtered at approximately 225 pounds of weight to determine the relationships of certain anatomical factors to conformation or plumpness.

When the hams were divided into nine groups according to increasing plumpness, no corresponding increase in average weights was found. Length of ham and length of leg decreased, whereas circumference of ham, thickness of ham fat, and thickness of back fat increased with plumpness fairly consistently. Also, there was a narrowing of the ratio of width of carcass through hams to length of hind leg and a decrease in length per unit weight of ham.

Semimembranosus, semitendinosus, rectus femoris, and adductor muscles did not consistently increase or decrease in weight with increase in ham plumpness. Proportion of semimembranosus muscle tended to increase and that of rectus femoris to decrease, but the sum of the percentages of the four muscles showed no trend. Percentages of separable fat and total edible meat increased with increasing plumpness, whereas the percentage of lean decreased slightly.

Percentages of separable fat and lean, thickness of ham fat, and ratio of edible to inedible portion of the ham had highly significant or significant correlations with plumpness. With separable lean the relation was inverse.

Only in the case of the rectus femoris muscle was weight significantly correlated with ham plumpness, the coefficient being negative. In percentage the relationship of the semimembranosus muscle was significant and that of the rectus femoris highly significant but inverse. Average thickness of back fat and ratio of width of carcass through hams to length of leg were relatively closely related to ham plumpness.

Correlations between percentage of separable fat and the percentages of the four muscles were highly significant or significant, and negative.

Close inverse relation existed between the percentages of separable lean and fat. The relationships of the four muscles to separable lean varied considerably, that of rectus femoris being closest. Percentage of separable lean was not a good index of the ratio of edible to inedible portion of the ham.

Correlations between weights of the four muscles and weight of ham were highly significant, although in no instance particularly high. Little relation occurred between thickness of ham fat or back fat and ham weight. Index of plumpness had much greater value than ham weight as an indicator of the proportion of edible meat.

Close, inverse, curvilinear relationship was found between length of ham per unit of weight and plumpness.

Multiple correlations indicated that variation in ham conformation was due more to combinations of certain factors than to single factors, but percentage of fat had more effect than any other single characteristic studied.

Weights of the semitendinosus and rectus femoris muscles were most closely related, and the former and the adductor muscle least closely related. With respect to percentages, the correlation between the semitendinosus and adductor muscles was highest and in general the latter was most closely related to the other three.

The reader is reminded that these results were obtained from a limited number of hogs of one breed. Therefore it seems a logical conclusion that possibly they are of more value for application to hams from hogs within a breed than to hams from hogs that vary as to breed.

A FOLIAR DIAGNOSIS STUDY OF THE EFFECT OF THREE NITROGEN CARRIERS ON THE NUTRITION OF *ZEA MAYS*¹

By WALTER THOMAS, *professor of plant nutrition*, and WARREN B. MACK, *professor of vegetable gardening, Pennsylvania Agricultural Experiment Station*

INTRODUCTION

In a recent paper (6)² the mode of nutrition of *Zea mays* growing on plots 1 to 9 of tier 1 of the Jordan fertility plots of the Pennsylvania Agricultural Experiment Station were compared by the method of foliar diagnosis (4). The carrier of nitrogen in the aforementioned plots is dried blood. Other carriers of nitrogen also are being tested in this long-continued experiment while the carriers of phosphorus and of potassium remain unchanged, the ratio $P_2O_5:K_2O=12:12.5$ being identical in all plots in the entire tier.

In a further examination of the uses to which the method of foliar diagnosis may be applied, the mode of nutrition of *Zea mays* subjected to treatments with complete fertilizers having dried blood as the carrier of nitrogen was compared with that with sodium nitrate as the carrier. A comparison of the mode of nutrition resulting from these treatments was then made with that of the treatment (manure+lime) giving the maximum yield, and the data were examined to determine the relationship of the foliar diagnosis to the yields. The results of this examination are reported in this paper.

Criticisms of the method of foliar diagnosis have been made that ignore some of the fundamental principles upon which the method is based, and have been explained in many of the writers' previous papers (4, 5, 6), in which emphasis has been placed upon the fact that the method is comparative just as diagnosis by means of the analysis of entire plants also is comparative (2). No physiological significance can be attributed to the foliar diagnosis of any one fertilizer (plot) considered alone and apart from the others (4). Accordingly, criticisms to the effect that the method does not take into account the action (if any) of rain water in removing soluble nutrients or of the possible return of nutrients from the plant to the soil are irrelevant, because neither the process of migration of the elements into the leaf from the stem or branch nor export from the leaf is under consideration in the method of foliar diagnosis, but only the amount of the element present in the chosen leaf at the moment of sampling. And, furthermore, it must be stressed that all the experiments carried out to test the validity of the method (4, 5) have been made under all sorts of climatic conditions.

The meteorological conditions will affect the mode or quality of nutrition. This influence will be indicated by its effect on the $N-P_2O_5-K_2O$ equilibrium and possibly also on the intensity of nutrition of the selected leaf. There is then a "chemism" due to the meteoro-

¹ Received for publication February 27, 1939. Paper No. 854 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

² Italic numbers in parentheses refer to Literature Cited, p. 313.

logical factor as well as a "chemism" due to the fertilizer itself. The mode of nutrition as expressed in foliar diagnosis of a given species in a given year is the resultant of both the meteorological factors and the fertilizer factor.

MATERIALS AND METHODS

The experiments were conducted on the Jordan fertility plots which were described previously (6). The plots studied in the present investigation together with their treatments and yields are listed in table 1.

TABLE 1.—*Plots studied, nitrogen sources, treatments, and yields*

Plot No.	Source of nitrogen	Treatment	N, P ₂ O ₅ , and K ₂ O equivalent applied to each plot	Symbol and ratio	Yield per plot	
					Grain	Stover
			<i>Pounds</i>		<i>Pounds</i>	<i>Pounds</i>
22.....	Manure.....	Manure+lime.....	$\left\{ \begin{array}{l} 8.88 \text{ (N)} \\ 4.00 \text{ (P}_2\text{O}_5) \\ 6.88 \text{ (K}_2\text{O)} \end{array} \right\}$	-----	770.8	408.9
9 and 17..	Dried blood.....	Dried blood.....	3.0	NPK, 5:12:25.....	520.6	213.0
		Superphosphate.....	6.0			
		Muriate of potash.....	12.5			
		Sodium nitrate.....	3.0			
26.....	Sodium nitrate.....	Superphosphate.....	6.0	(3N) PK, 18:12:25..	705.5	359.5
		Muriate of potash.....	12.5			
		Dried blood.....	9.0			
		Superphosphate.....	6.0			
11 and 21..	Dried blood.....	Muriate of potash.....	12.5		527.6	233.2
		Sodium nitrate.....	9.0			
		Superphosphate.....	6.0			
		Muriate of potash.....	12.5			
28.....	Sodium nitrate.....	Superphosphate.....	6.0		722.7	372.6
		Muriate of potash.....	12.5			

The method of leaf sampling was similar to that already described (4, 5, 6). The third leaf from the base was sampled from each plant in a row lengthwise across the plot. Successive rows were sampled at the dates recorded in table 2.

PRESENTATION OF RESULTS

The plot numbers, treatments, and yields are shown in table 1. The percentages of N, P₂O₅, and K₂O in the third leaf from the base of the stalk at the various sampling dates are given in table 2, together with their milligram-equivalent values and the composition of the NPK unit representing the equilibrium between N-P₂O₅-K₂O at the moment of sampling.

DISCUSSION AND INTERPRETATION OF RESULTS

In the following discussion, for the sake of simplicity and to avoid repetition, the treatment or treatment symbol, or both, either with or without the plot number, is used to indicate the composition of the third leaf from the base of plants growing on the plots receiving the treatment designated.

TABLE 2.—Percentages of N, P₂O₅, K₂O, CaO, and MgO in the dried foliage, milligram-equivalents, and the composition of the NPK unit for plants grown under the different fertilizer treatments

Date	6 TONS MANURE+CAO (PLOT NO. 22)									
	Mineral content of dried foliage					Milligram-equivalents				
	N		P ₂ O ₅		K ₂ O		CaO		MgO	
	(M)%	Percent	(M)%	Percent	(M)%	Percent	(M)%	Percent	(M)%	Percent
July 6	2.530	0.558	2.740	0.680	2.793	0.690	2.757	0.680	2.757	0.680
July 21	2.910	0.874	3.296	0.717	3.160	0.717	24.280	58.362	24.280	58.362
Aug. 8	2.640	0.810	3.242	0.760	3.381	0.760	21.473	44.559	21.473	44.559
Aug. 26	2.540	0.550	2.163	0.551	3.263	0.551	23.263	46.072	23.263	46.072
NPKN AS DRIED BLOOD (PLOTS NOS. 9 AND 17)										
July 6	2.920	0.415	3.906	0.457	1.590	0.457	17.554	83.208	17.554	83.208
July 21	2.380	0.465	3.880	0.534	1.862	0.534	19.711	81.589	19.711	81.589
Aug. 8	2.500	0.562	3.054	0.773	1.773	0.773	23.772	65.050	23.772	65.050
Aug. 26	2.460	0.645	2.776	0.827	1.740	0.827	27.283	59.134	27.283	59.134
NPKN AS NaNO ₃ (PLOT NO. 26)										
July 6	3.410	0.412	3.837	0.471	1.933	0.471	243.474	81.728	243.474	81.728
July 21	2.960	0.488	3.643	0.464	2.313	0.464	211.344	77.596	211.344	77.596
Aug. 8	2.740	0.566	2.946	0.553	2.237	0.553	23.942	62.750	23.942	62.750
Aug. 26	2.330	0.570	2.267	0.525	2.207	0.525	166.362	48.287	166.362	48.287
(3N) PKN AS DRIED BLOOD (PLOTS NOS. 11 AND 21)										
July 6	2.860	0.470	4.306	0.403	1.26	0.403	204.204	91.718	204.204	91.718
July 21	2.565	0.479	3.732	0.404	1.52	0.404	183.141	70.502	183.141	70.502
Aug. 8	2.615	0.496	2.973	0.568	1.56	0.568	186.711	63.327	186.711	63.327
Aug. 26	2.740	0.610	2.752	0.606	1.68	0.606	195.631	58.618	195.631	58.618
(3N) PKN AS NaNO ₃ (PLOT NO. 28)										
July 6	3.420	0.426	3.406	0.508	1.994	0.508	244.188	72.549	244.188	72.549
July 21	3.070	0.511	3.585	0.534	2.100	0.534	219.198	76.360	219.198	76.360
Aug. 8	2.860	0.536	2.865	0.471	2.146	0.471	204.204	61.024	204.204	61.024
Aug. 26	2.800	0.610	2.791	0.480	2.150	0.480	199.920	59.448	199.920	59.448
Composition of the NPK unit										
						X	Y	Z		
						$\left(100 \times \frac{E_x}{S}\right) \frac{N}{N}$	$\left(100 \times \frac{E_y}{S}\right) \frac{P_2O_5}{P_2O_5}$	$\left(100 \times \frac{E_z}{S}\right) \frac{K_2O}{K_2O}$		
						75.652	6.831	17.518		
						71.481	8.353	20.166		
						74.028	8.472	17.500		
						72.342	9.280	18.378		
						67.455	5.670	26.875		
						62.700	7.258	30.041		
						67.211	8.777	24.013		
						67.024	10.411	22.565		
						71.060	5.087	23.853		
						68.268	6.668	25.065		
						69.294	8.480	22.224		
						69.678	10.098	20.925		
						65.256	5.434	20.309		
						64.737	7.162	28.102		
						68.892	7.741	23.367		
						69.834	9.240	20.925		
						72.945	5.383	21.672		
						69.110	6.815	24.075		
						70.929	7.875	21.196		
						70.105	9.048	20.846		

NITROGEN

At the first date of sampling (July 6) the content of nitrogen of the leaf was greatest in the treatment giving the highest yield (manure + lime, plot No. 22), and was followed in descending order both with respect to nitrogen content and yields by (3N)PK (plot No. 28); NPK (plot No. 26), in which nitrogen is in the form of

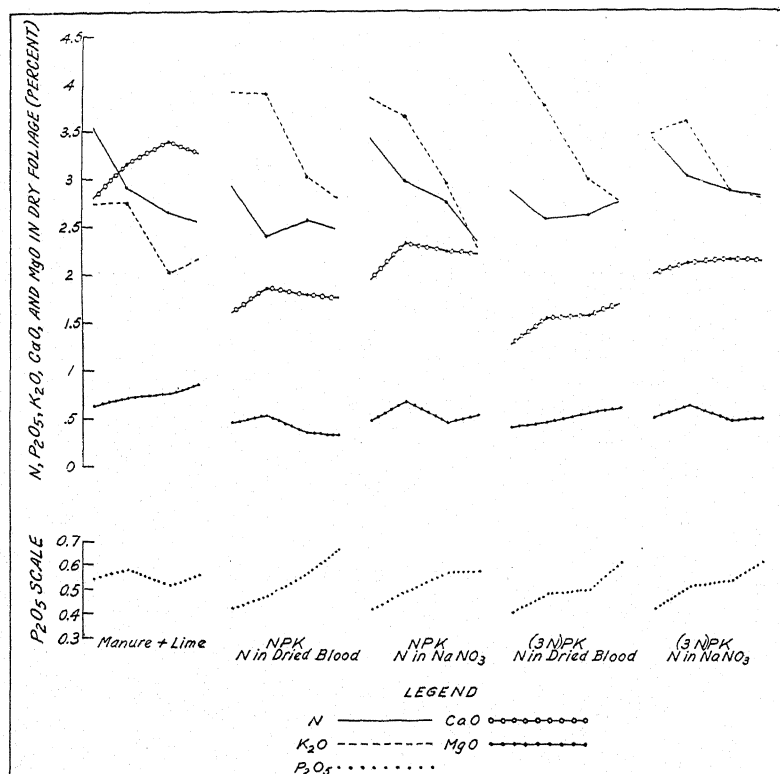


FIGURE 1.—The percentages (dry basis) of nitrogen, phosphoric acid, potash, lime, and magnesia in the third leaf of plants growing in plots receiving the treatments indicated are plotted as ordinates and dates of sampling as abscissae. The dates of sampling (July 6, July 21, August 8, August 25) are indicated by the four dots in each curve.

sodium nitrate; and then by NPK (plots Nos. 9, 17) and (3N)PK (plots Nos. 11, 21) in which nitrogen is in the form of dried blood. At this date, then, there is a relationship between the nitrogen in the leaf and the yields. But this superiority in nitrogen content of manure + lime (No. 22) does not continue with advancing age of the leaf, for, as the relative steepness of the graphs (fig. 1) indicates, the great demand for nitrogen relative to supply of the plants growing on manure + lime (No. 22) causes the graph of this treatment to fall below that of the next highest yielding plot (3N)PK (No. 28) during the remainder of the growth cycle. The nitrogen graph of manure + lime, however, is still above those resulting from the

dried blood treatments, except towards the end of the last period in (3N)PK (Nos. 11 and 21).

Nitrogen in dried blood, then, supplied nitrogen at a lower level of nutrition than did manure + lime or sodium nitrate—an indication of slow availability. Not only do the graphs indicate slow availability of the nitrogen of dried blood in these plots which have never received lime additions, but also the ascent of the graphs indicate in addition that the utilization also is poor.

PHOSPHORIC ACID

At the first date of sampling (July 6) the P_2O_5 content of the selected leaves from the highest yielding treatment, manure + lime (plot No. 22), was the greatest. This superiority of manure + lime continued up to the middle of the second period (about August 1). At the latter date an accumulation of P_2O_5 , which occurs both in the sodium nitrate and dried blood plots, causes the graphs of these treatments to rise above that of manure + lime (plot No. 22).

During the first period the differences in the P_2O_5 content of the selected leaf of plants receiving the dried blood and sodium nitrate treatments are insignificant. With advancing age greater differences are noticed, but no regularity is apparent. Accumulation with age, as shown by the ascent of the graphs, occurs in all treatments, and is especially marked in the graphs from the lower application of dried blood. Accumulation of P_2O_5 is insignificant in the manure treatment.

Accumulation of P_2O_5 with the advancing age of the leaf is an abnormal behavior. In plants hitherto examined, the content of P_2O_5 decreased with the advancing age of the leaf.

When nitrogen is plotted against phosphoric acid in the manner described in an earlier paper (3), and as shown in figure 2, it is found that relative to the optimum graph indicated by the line $y=2.109x+0.488$, P_2O_5 is too low relative to N during the early period, and too high during the last period in all of the plots examined in the experiments reported in this paper.

POTASH

During the greater part of the first two periods (July 6–21, and July 21–August 8) the potash content was highest in the chosen leaves of plants growing on the dried-blood plots (Nos. 11, 21) which received the highest amount of nitrogen and which gave next to the lowest yields of the treatments considered in this paper. These are followed in descending order of potash content of the leaf during this period by NPK (Plot Nos. 9 and 17), NPK (plot No. 26), (3N)PK (plot No. 28), and manure + lime (plot No. 22). If the dried blood treatments are considered together, the potash content of the selected leaves during this period is in the reverse order of the respective yields. During the last period, August 8–25, there was little difference between the content of the leaves growing on the dried-blood plots, but because of small demand relative to supply in (3N)PK (plot No. 28) receiving sodium nitrate, the potash content of the leaf was slightly above that of those receiving dried blood. In effect “luxury consumption” of potash occurred in the plots which have never received lime. This excess absorption of potash has been harmful to yields—an illustration of the reciprocal effects between calcium and potassium (1).

LIME

The graph for CaO is highest throughout the whole period in the limed manure plot (No. 22), followed in descending order of position

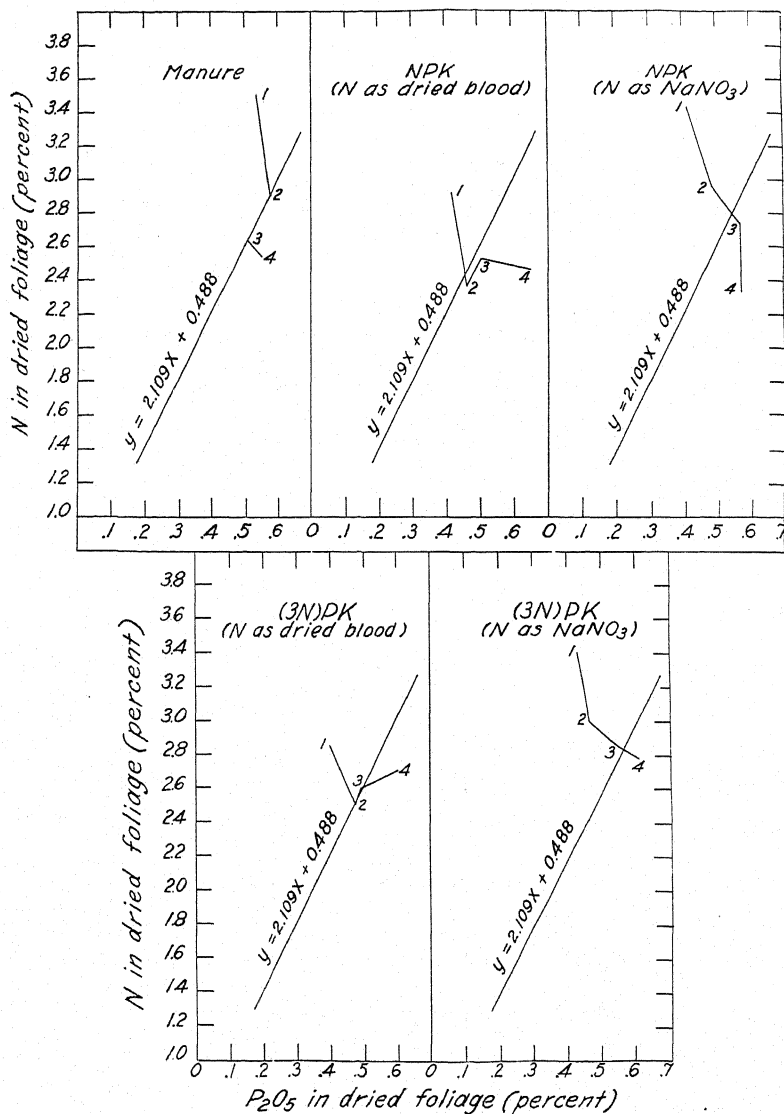


FIGURE 2.—Relation of the percentages of nitrogen and phosphoric acid in the third leaf of plants growing on plots receiving the treatments indicated, at the different dates of sampling: 1, July 6; 2, July 21; 3, August 8; and 4, August 25.

by NPK (plot No. 26), (3N) PK (plot No. 28), and then by the dried-blood plots NPK (Nos. 9 and 17) and (3N)PK (Nos. 11 and 21). The fact that the graphs of No. 26 and No. 28 with sodium nitrate treatments are always well above the corresponding graphs from the

dried blood treatments is an indication of the greater availability of calcium under sodium nitrate treatments.

Because of the extreme sensitivity of the method of foliar diagnosis the influence of one element on the absorption of another is readily detected. Because of this influence there can be no a priori right to use the quantity of an element in a fertilizer as a variable in the interpretation of results of the effects of fertilizers. An element may be readily absorbed and utilized, but nevertheless may have an injurious effect on growth, because of the effect on the absorption of another element. An illustration occurs in the Jordan plots where added potash has decreased the yields. Thus NK (plot No. 7) has given lower yields than N (plot No. 2), and the foliar diagnosis of these plots (6) shows that the effect is the result of the depression of the absorption of CaO in the leaves of plants growing on NK (plot No. 7) (plot No. 7) which is below not only the N treatment, (plot No. 2), but even below that of the check (plot No. 1).

In the present experiments the addition of nitrogen as sodium nitrate (plots No. 26 and 28) by reducing the content of potash in the selected leaves nearer to that of the optimum, manure+lime (plot No. 22), has resulted in an elevation of the CaO graph nearer to that of the optimum, with improved yields.

MAGNESIA

The MgO graphs in general bear the same relation to one another as do the CaO graphs.

INTENSITIES OF NUTRITION AND THE COMPOSITION OF THE NPK UNITS

The interpretation of the data is simplified if only the three plastic elements nitrogen, phosphorus, and potassium are taken into consideration. There are logical grounds for this position; for although there may be no a priori right to relate the composition of a leaf based on the entities nitrogen, phosphoric acid, and potash alone, nevertheless field tests are conducted on the assumption that the other factors for the plots to be compared are either equal or nearly so. Hence it is apparent that if, in any experiment, this assumption is not valid, then the basis for the calculation of probabilities is subject to question. The agronomist then is faced with the dilemma that he must either relate differences in yield to the nutrition of the plant as in the method of foliar diagnosis or draw conclusions on the basis of probabilities computed with assumptions of which the validity may be questionable. If, then, the three elements which are plastic in nutrition and upon which the cost of fertilizers is based, are considered, a fertilizer can intervene in the nutrition of a plant to cause a change in the quantity (or intensity of nutrition) or in the quality (physiological ratios of the elements), or in both simultaneously. The former (intensity) is the sum of the plastic elements ($N + P_2O_5 + K_2O$) expressed as the percentage of the dry weight of the leaves, and the quality is best expressed as the NPK-unit which represents the equilibrium between $N - P_2O_5 - K_2O$ in the chosen leaf at the moment of sampling. The NPK-unit is derived (4, 6) by converting the percentage values into milligram equivalents and then finding the proportion which each

of these bears to the milligram-equivalent total. The results are then multiplied by 100 to avoid fractional quantities.

The intensities of nutrition and also the composition of the NPK units from one sampling date to another are shown in the fifth, and in the last three columns of table 2, respectively. The NPK units are plotted in trilinear coordinates in figures 3 and 4.

THE INTENSITIES OF NUTRITION

The higher applications of nitrogen either as dried blood or as sodium nitrate had little effect on the intensity of nutrition, except at the last sampling; at this date the intensity was much higher in the dried blood

plots which received the higher nitrogen application. With one exception (NPK, No. 26), on August 25, the intensity resulting from the manure + lime treatment (No. 22) was lower than any considered. This reduction in intensity was the result of reduction in the absorption of potash.

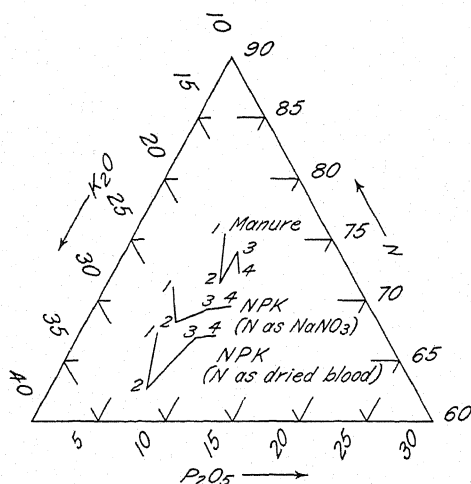


FIGURE 3.—The composition of the NPK units of the third leaf of plants growing on the manure and NPK plots, at the different dates of sampling: 1, July 6; 2, July 21; 3, August 8; and 4, August 25.

similar displacements in direction in the equilibrium between $N-P_2O_5-K_2O$, although the magnitudes of these displacements are unequal.

During the first period all treatments result in a decrease in the nitrogen in the NPK unit, indicating that at this early period the fertilizer is unable to overcome the declining tendency of the medium (soil and meteorological factors) with respect to nitrogen, with the advancing age of the leaf.

As the season advances all graphs reverse their direction, and the quota-part of nitrogen now increases at the expense of the potash in the NPK unit, indicating that the declining tendency of the medium with respect to nitrogen has been checked and even reversed. In manure (No. 22) and (3N)PK (No. 28) the quota-part of nitrogen in the unit again decreases.

The P_2O_5 in the NPK unit increases with advancing age of the leaves in all treatments. This increase is made at the expense of the nitrogen in the first period (July 6–21); at the expense of the potash in the second period (July 21–August 8) and either at the expense of

COMPOSITION OF THE NPK UNITS

The changes in the $N-P_2O_5-K_2O$ equilibrium from one sampling to another are best followed from the graphs showing the changes in the NPK unit (figs. 3 and 4).

During the first two periods (July 6–21, and July 21–August 8) the different treatments have produced

the nitrogen (manure+lime, plot 22, and (3N)PK, plot 28) or of the potash in the third period (August 8-25).

The qualitative pattern is thus in general similar for all treatments. Quantitatively, however, differences occur and are best considered by means of the mean values of the NPK units during the cycle (fig. 5).

THE MEAN INTENSITIES AND THE MEAN NPK UNITS

The mean intensities and the mean NPK units represent the mean of the respective values during the growth cycle given in table 2 for the intensities and NPK units, respectively, at the four sampling dates; these are shown in table 3. The values of the mean NPK units can be represented by a point on a trilinear diagram (fig. 5), and each point represents the center of gravity of the respective diagrams of figures 3 and 4.

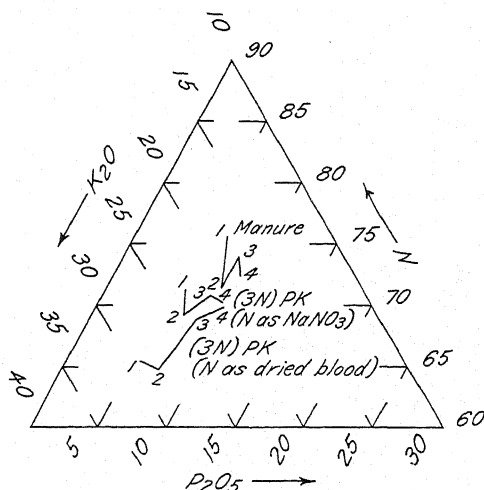


FIGURE 4.—The composition of the NPK units of the third leaf of plants growing on the manure and (3N) PK plots, at the different dates of sampling: 1, July 6; 2, July 21; 3, August 8; and 4, August 25.

TABLE 3.—The relation of the mean intensities of nutrition and the composition of the mean NPK-unit to yields of grain

Plot No.	Treatment	Source of nitrogen	Mean intensity	Composition of the mean NPK unit	Yield of grain per plot
					Pounds
22	Manure+CaO.....	Manure.....	5.88	73.38:8.23:18.39	770.8
28	(3N) PK.....	NaNO ₃	6.73	70.77:7.28:21.95	722.7
26	NPK.....	do.....	6.54	69.57:7.58:22.84	705.5
11, 21	(3N) PK.....	Dried blood.....	6.63	67.18:7.39:25.42	527.6
9, 17	NPK.....	do.....	6.50	66.08:8.03:25.88	520.6

THE MEAN INTENSITY

The values for the mean intensity are slightly higher for the higher applications of nitrogen than for the lower. As before noted, the values of the manure+lime treatment are much lower than for the other plots.

THE MEAN NPK-UNITS

The composition of the mean NPK-units, on the other hand, is distinctly affected by the source of nitrogen. Relative to the dried blood treatments, sodium nitrate has resulted in an increase in the nitrogen, principally at the expense of the potash.

Increased applications of nitrogen either as dried blood or as sodium nitrate also have resulted in a decrease in the P₂O₅, the effect being greater with the former than with the latter.

described, and the latter magnitudes are shown in trilinear coordinate diagrams. In relation to the optimum treatment (manure+lime), the intensities of the other treatments discussed in this paper are too high. Nitrogen and phosphoric acid in the NPK unit are too low, and potash is too high.

The positions of the mean values during the growth cycle of the NPK-units for a particular treatment in relation to that of the optimum treatment show a definite correlation with yields.

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No. 5

COMPOSITION OF THE VARIOUS PARTS OF THE OAT PLANT AT SUCCESSIVE STAGES OF GROWTH, WITH SPECIAL REFERENCE TO THE FORMATION OF LIGNIN¹

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INTRODUCTION

The present paper is one of a series (9, 36, 38)³ from this Bureau dealing with the chemical composition of certain crop plants at different stages of growth and development. The object of these studies was to determine the nature and percentage of the more important components of the plant, both structural and nonstructural, as well as to ascertain their possible interrelationships, with a view to learning more about the formation of lignin by the growing plant and the mechanism involved in the process of lignification.

This paper reports a study of the composition of several parts of the oat plant at different stages of development. Samples were taken at weekly intervals, beginning 1 week after the plants emerged and continuing until they became mature and senescent. Inasmuch as growth is a dynamic process, results giving only the percentage composition of a plant at different stages of development may be deceptive and misleading, for an increase in the percentage of one component may not necessarily indicate an actual increase in the absolute quantity of this constituent, but may be due rather to a diminution or translocation of some other component or components. Accordingly, in this investigation not only were the percentages of the more important constituents determined, but their absolute quantities were also ascertained at successive stages of development of different parts of the oat plant. In view of the fact that the primary object of this investigation was to study the development of lignin in the plant, no chemical examination of the grain at different stages of development was undertaken.

REVIEW OF LITERATURE

A review of the literature relative to the composition of certain plants at different stages of growth was presented in a previous paper (38). In this paper, a review will be given of the more recent literature on the subject, together with a résumé of the older publications that are of particular interest in connection with the results of the present investigation.

¹ Received for publication May 6, 1939. Investigation conducted in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Idaho Agricultural Experiment Station. A résumé of this paper was presented on April 6, 1939, at the ninety-seventh meeting of the American Chemical Society, held at Baltimore, Md.

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³ Italic numbers in parentheses refer to Literature Cited, p. 363.

About 90 years ago the American agricultural chemist Norton (32), working in the laboratory of the Agricultural Chemistry Association of Edinburgh, Scotland, made an extensive study of the composition of the oat plant at different stages of growth. He determined the percentage of ash as well as the chemical composition of the ash in the culms, leaves, knots (culm nodes), chaff, and grain. He found that the percentage of ash in the leaf and in the chaff increased steadily as the plants grew older, while in the stalks, after an initial increase, it decreased as the plants matured. The percentage of ash in the knots was found to be greater than that in the entire stalk. He also reported that there was a difference in the percentage of ash yielded by different parts of the same stalk. There was not, however, a regular gradation in the percentage of ash from the top of the stalk downward.

Collins and Spiller (7) reported that green oat straw may contain approximately 6 percent of sugar, mostly invert sugar, while fully mature straw contains practically no sugar.

Rogozinski and Starzewska (41) determined the percentage of ash, crude fiber, pentosans, and methoxyl at successive stages of growth of the oat plant. Lignin was not determined directly, but was calculated from the percentage of methoxyl found, the assumption being that the percentage of methoxyl in lignin from cereal straw is 13.50 and that lignin is the only methoxyl-containing substance in the oat plant. It is now known, however, that this assumption is not true, so that their lignin values cannot be accepted without reserve. Their results showed that the percentage of crude fiber, pentosans, methoxyl, and lignin increased as the plant grew older, while the ash decreased.

Fagan and Watkin (13) found that the crude protein in the leaves and culms decreased rapidly with the increase in the age of the oat plants. The percentage of ash in the culms at first increased, and then decreased somewhat as the plants matured. The percentage of ash in the leaves decreased steadily with the increase in the age of the plants.

Wagner (47) reported that during the later stages of development of the oat plant there was a constant decrease in the weight of the leaves and culms. This, however, did not apply to the top growth of the plants as a whole.

Phillips and Goss (38), in studying the composition of the leaves and stalks of the barley plant at successive stages of growth, found that after an initial increase the percentage of ash and nitrogen declined steadily as the plant matured. The percentage of methoxyl in the original and in the extracted plant materials increased with the age of the plant. The percentage of alcohol-benzene and cold- and hot-water extractives declined, though not regularly, as the plant became older. The percentage of uronic acids increased somewhat during the early development of the plant, and then declined as the plant matured. The percentage of the furfural-yielding components, as well as the percentage of pentoses calculated as pentosans, increased as the plant matured. The percentage of cellulose, Cross and Bevan cellulose, the furfural-yielding fraction of Cross and Bevan cellulose, and lignin, as well as the percentage of methoxyl in the lignin, also increased regularly as the plants developed and matured. No direct evidence was obtained that the barley plant synthesizes lignin from cellulose, pentoses, or pentosans.

Norman (29) and Norman and Richardson (30), who studied the composition of ryegrass (western wolfs, *Lolium multiflorum*) at different stages of development, found that the percentage of cellulose, xylan in the Cross and Bevan cellulose, and lignin increased progressively with the age of the plant. No direct determination of the polyuronide hemicelluloses was made, but from the percentage of furfural found it was concluded that they did not increase regularly and were lower in the mature grass than in the young grass. A water-soluble fructosan was found in considerable quantity in the younger plants. The maximum percentage of the fructosan was attained at about the time of full emergence of the head, and it then decreased as the plant matured.

Many suggestions are found in the literature concerning the nature of the parent substance and the possible mechanism involved in the synthesis of lignin by the plant. Most of these suggestions are either purely speculative or are based on indirect or fragmentary evidence.

Klason (20) believes that lignin is formed by the plant from pentoses or methyl pentoses and that coniferyl alcohol (or coniferyl aldehyde) is an intermediate product formed in the process. This view is also shared by Von Euler (12).

Rassow and Zschenderlein (40) point out that plant materials with a high content of lignin have a low content of pentosans, and vice versa. They suggest, therefore, that lignin, at least in part, is built up by the plant from pentosans.

According to Odén (33), pentoses are first converted into methyl pentoses, and these are then condensed to form benzofurane derivatives from which lignin is synthesized.

Green (17), Cross, Bevan, and Beadle (8, pp. 177-181), König and Rump (21), and Fuchs (14, 15) have suggested that lignin is formed by the plant from cellulose.

Schrauth (45) and Jonas (19) believe that soluble carbohydrates, such as pentoses and hexoses, particularly glucose, are utilized by the plant in the formation of lignin. Casparis (6) believes that the carbohydrates of the cell wall are the precursors of lignin.

According to an older view of Wislicenus (48), lignification is a process whereby the sum total of the dissolved hydrosols in the cambial sap is deposited upon the cellulose fiber. In a more recent publication (49), however, he suggests that while glucose is utilized by the plant in the formation of cellulose, fructose is used similarly in the synthesis of lignin.

The possibility of a biogenetic relationship between pectin and lignin has been considered by several investigators. It has been observed by Candlin and Schryver (5) that pectin is found rather abundantly in young and nonlignified tissues, while plants rich in lignin are relatively free from pectin. Ehrlich (11) isolated from a hydro-pectin of flax a fraction that resembled lignin in certain respects. Accordingly, he suggested that pectin is the precursor of lignin. The evidence for this, however, is not convincing.

Buston (4), in his studies of the process of lignification of the rose plant, proved definitely that lignin is not produced at the expense of the pectin. While in the later stages of the development of the plant, the lignin content increased considerably, the absolute quantity of the pectin did not decrease but actually increased. He concludes,

therefore, that "lignin does not arise from pectin, but more probably from some carbohydrate related to the glucosan-xylan series with members of which series it is usually associated in the plant."

According to Zherebov (50), primary lignin contains no methoxyl groups, and he therefore considers that lignification is a process by which methoxyl groups are accumulated. While lignin from a fully mature annual plant contains a higher percentage of methoxyl than lignin from an immature plant, it has been repeatedly demonstrated in this laboratory that lignin from even a very young plant always contains some methoxyl.

MATERIAL AND METHODS OF ANALYSIS

The oat plants (*Avena sativa* L., Victory variety) used in this investigation were grown under irrigation at Aberdeen, Idaho. The type of soil in that locality is described as an Aberdeen silty clay loam. The oat seeds were sown on April 24, 1936, and the young plants emerged on May 6. The age of the plants was calculated from the day they emerged. The first harvest took place in the morning of May 13 and subsequent samplings were made at weekly intervals, at approximately the same time of the day. The plants were taken up by the roots, freed from soil, and counted. The roots were then cut off from the culms, freed from the mother seeds, thoroughly washed in running water, and freed as nearly as possible from adhering soil. The culms and roots were dried in a steam oven at 50° C. and then spread out in the laboratory and allowed to air-dry. During heading time (when the plants were 63 days old and at each subsequent harvest period until maturity), the heads were cut off, and these were separated into grain, chaff, and rachises and branches of the panicles. With the exception of the samples from the first two harvest periods, all analyses were made on air-dried materials. Separate samples were taken for moisture and ash determinations, and the analytical data were then calculated on the moisture-free and ash-free basis. For most of the analyses, the plant materials were ground in a Wiley mill fine enough to pass through a 60-mesh sieve. Plant material ground to pass through an 80-mesh sieve was used for the determination of the percentages of material extracted by alcohol-benzene, cold and hot water, and 1 percent of hydrochloric acid as well as for the determination of lignin.

The following determinations were made:

Moisture.—A weighed air-dry sample of the material was dried at 105° C. to constant weight, and the loss in weight was determined.

Ash.—Ash was determined by igniting a weighed sample in an electric muffle furnace at 600° C. and weighing the inorganic residue.

Nitrogen and crude protein.—All nitrogen determinations were made by the Kjeldahl-Gunning-Arnold method (2, p. 25). The nitrogen values were also calculated as percentages of crude protein ($N \times 6.25$).

Methoxyl in original plant material.—The percentage of methoxyl in the original unextracted plant material was determined by the method described by one of the writers in a previous publication (35). The methyl iodide was absorbed in pyridine.

Methoxyl in extracted plant material.—The plant material was successively extracted with a 1:2 alcohol-benzene solution, cold water, hot water, and a 1-percent hydrochloric acid solution, by the pro-

cedure hereinafter described for determining these extractives, and the percentage loss in weight due to these extractions was determined. The percentage of methoxyl in the extracted plant material was then determined by the method used for the determination of methoxyl in the original unextracted plant material, and the result was calculated on the basis of the original unextracted moisture-free material as well as on the ash-free basis. The extraction operation removed alcohol-benzene-soluble and water-soluble methoxyl-containing substances as well as any methoxyl groups occurring as methyl esters of organic acids, such, for example, as are found in the pectins. The percentage of methoxyl found in the residual extracted plant material represents essentially lignin methoxyl, together with some firmly bound methoxyl (present in etherlike combination) occurring in other components of the plant.

Alcohol-benzene extractives.—These were determined by extracting a weighed sample of the material in a Soxhlet extractor for 30 hours with a 1:2 alcohol-benzene solution, and ascertaining the loss of weight.

Cold-water extractives.—To the weighed sample of the dry material extracted with alcohol-benzene solution, distilled water was added in the proportion of 150 cc. to 1 gm. of sample, and the mixture was allowed to stand at room temperature, with frequent stirring, for 48 hours. The insoluble material was filtered off and dried at 105° C., and the loss in weight was determined. The filtrate was added to a 2-liter volumetric flask and made up to the mark with distilled water (solution A), and 1,000 cc. of this solution was transferred portionwise to a weighed porcelain dish, and evaporated to dryness on the steam bath. The residual material in the dish was ignited in an electric muffle at 600°, the weight of ash was determined, and the total ash in solution A was calculated. Two 250-cc. samples of solution A were taken for nitrogen determinations by the Kjeldahl-Gunning-Arnold method (2, p. 25), and the total nitrogen found was calculated as grams of crude protein ($N \times 6.25$). The loss in weight due to extraction with cold water, previously determined, minus the combined weights of ash and crude protein in solution A was calculated as percentage of the original moisture-free and ash-free material.

Hot-water extractives.—To the weighed sample of the dry residue from the cold-water extraction, distilled water was added in the proportion of 150 cc. of water to 1 gm. of sample, and the mixture was boiled under a reflux condenser for 3 hours. The insoluble material was filtered off and dried at 105° C., and the loss in weight was determined. The combined weights of ash and crude protein in the filtrate were determined as described in the preceding paragraph and deducted from the loss in weight resulting from the hot-water extraction. The result obtained was calculated as percentage of the original moisture-free plant material and also as percentage of the original moisture-free and ash-free material.

One-percent hydrochloric acid extractives.—The weighed sample of the plant material that had been successively extracted with alcohol-benzene solution, cold water, and hot water, as described above, was treated with a 1-percent hydrochloric acid solution in the proportion of 150 cc. of acid solution to 1 gm. of plant material and boiled under a reflux condenser for 3 hours. The remainder of the procedure was the same as that described under cold-water extractives.

Total extractives.—The total extractives are the sum of the percentages of the alcohol-benzene, cold-water, hot-water, and 1-percent hydrochloric acid extractives.

Uronic acid anhydrides.—The uronic acid anhydrides were determined in the unextracted material according to the procedure recommended by Dickson, Otterson, and Link (10), as modified slightly by Phillips, Goss, and Browne (37). The uronic acids are present chiefly in the pectins and in the hemicelluloses.

Total furfural.—Furfural was determined in the original unextracted plant material by the method of the Association of Official Agricultural Chemists (2, pp. 344-345), which was the method developed by Tollens and his coworkers, particularly Kröber (22). The weight of furfural corresponding to the weight of furfural-phloroglucide was obtained from Kröber's table (2, pp. 641-643).

Pentosans.—Pentosans represent the difference between the total furfural and the furfural derived from the uronic acids calculated as percentage of pentosans.

When uronic acids are boiled with 12-percent hydrochloric acid, as in the determination of pentosans, they are decomposed into furfural, carbon dioxide, and water. While the yield of carbon dioxide is quantitative (this fact is the basis of several methods described in the literature for the quantitative estimation of uronic acids), the yield of furfural is not. Lefèvre and Tollens (25) distilled glucuronic acid anhydride and derivatives of this compound with 12-percent hydrochloric acid, under the experimental conditions prescribed for the determination of pentosans, and showed that the weight of furfural phloroglucide produced amounted to one-third of the weight of glucuronic acid anhydride present. By substituting the weights of furfural taken from Kröber's table (2, pp. 641-643) for the weights of furfural phloroglucide obtained by Lefèvre and Tollens in 12 determinations and on 4 different products, it was calculated that their average yield of furfural amounted to 19.11 percent of the weight of glucuronic acid anhydride, or the ratio of furfural to glucuronic acid anhydride was 1 to 5.23, Norris and Resch (31) distilled euxanthic acid with 12-percent hydrochloric acid and found that the average yield of furfural in 8 determinations amounted to 21.48 percent of the weight of the glucuronic acid anhydride so that the ratio of furfural to glucuronic acid anhydride was 1 to 4.66. The mean value of the two ratios is 1 to 4.95. The yield of furfural from galacturonic acid anhydride is given by Norris and Resch (31) as 23.50 percent of the weight of the uronic anhydride so that the ratio of furfural to galacturonic acid anhydride is 1 to 4.26. Accordingly, to determine the percentage of furfural corresponding to the uronic acids present, the percentages of the latter were divided by the factor 4.60 (mean value of 4.95 and 4.26. Thus,

$$\frac{\text{percentage of uronic acid anhydride}}{4.60} = \text{percentage of furfural derived}$$

from the uronic acids. In this calculation it is assumed of course (1) that in lignified plant material both glucuronic and galacturonic acid are present and in the proportion of 1 to 1, and (2) that the yield of furfural from glucuronic acid is not affected by the presence of galacturonic acid, pentoses, hexoses, etc., and (3) that the yield of furfural from galacturonic acid is not affected by the presence of glucuronic acid, pentoses, hexoses, etc. However, it is believed that a reasonably

close approximation of the yield of furfural derived from the uronic acids is obtained by the above-described method. In the present state of knowledge of the composition of the plant cell wall, it is not possible to obtain results of greater precision and accuracy. The difference between the percentage of total furfural and the percentage of furfural derived from the uronic acids when multiplied by the factor 1.71 gives the percentage of pentosans, that is, the pentosan fraction associated with the Cross and Bevan cellulose plus that present in the polyuronide fraction.

Cross and Bevan cellulose.—The weighed sample contained in a 60-cc. sintered-glass crucible (porosity 2 to 3) was placed in a 150-cc. beaker, and a boiling 1 to 2 alcohol-benzene solution was added to within one-half inch of the top of the crucible. As the solvent drained into the beaker, more alcohol-benzene solution was added until the level of the solvent in the beaker was the same as that in the crucible. The beaker, covered with a watch glass, was then placed on the steam bath and allowed to remain there for one-half hour. The temperature of the steam bath was so regulated that the alcohol-benzene solution in the beaker was just below its boiling point. The solvent in the crucible was removed with suction, and the extraction with hot alcohol-benzene solution was repeated twice, and then was extracted with hot 95-percent ethanol. The extracted plant material in the crucible was washed with boiling water, the excess water was removed with suction, and the crucible and contents were allowed to cool to room temperature. The plant material was then alternately chlorinated and digested with a 2-percent sodium sulfite solution, then bleached with a 0.1-percent potassium permanganate solution, and finally treated with a sulfurous acid solution, as described by one of the writers in a previous paper (35). The bleached Cross and Bevan cellulose was removed from the crucible with the aid of a glass rod, and the cellulosic material, as well as the nearly empty crucible, was placed in an 800-cc. beaker. Three hundred cubic centimeters of boiling water was added to the beaker, and the mixture was digested on the steam bath for 2 hours. The crucible was removed from the beaker, and any adhering cellulosic material was washed into the beaker. The contents of the beaker were then added portionwise to the crucible, filtered, washed successively with hot water, 95-percent ethanol, and ether and finally dried for 4 hours at 105° C. and weighed. The percentage of ash in a portion of the Cross and Bevan cellulose was determined, and the percentage of ash-free Cross and Bevan cellulose was then calculated.

Furfural and xylan from Cross and Bevan cellulose.—This determination was made by the same method used for the quantitative estimation of the total furfural in the plant materials. The percentage of furfural was calculated on the basis of ash-free Cross and Bevan cellulose. From the percentage of furfural thus found, the percentage of xylan in the ash-free Cross and Bevan cellulose was calculated. The xylan in the Cross and Bevan cellulose was also calculated on the basis of the original moisture-free and ash-free material.

Cellulose.—The percentage of xylan in the Cross and Bevan cellulose was deducted from the percentage of Cross and Bevan cellulose, and the result was expressed as percentage of cellulose in the original

moisture-free and ash-free material. Thus if X equals the percentage of xylan in the Cross and Bevan cellulose and Y the percentage of Cross and Bevan cellulose, then $Y - \frac{X \times Y}{100}$ = percentage of cellulose.

Reducing and nonreducing sugars.—The sugars were extracted from the weighed sample (10 gm. plus 3 gm. of calcium carbonate), the alcohol was removed, and the sugar solution was cleared according to the method of the Association of Official Agricultural Chemists (2, pp. 134–135). The reducing sugars were determined by the method of Munson and Walker (2, p. 479), and the results were calculated as percentage of dextrose in the original moisture-free and ash-free material.

The nonreducing sugars were determined in an aliquot of the sugar solution by the method of the Association of Official Agricultural Chemists (2, pp. 341–342), and the results were calculated as percentage of sucrose in the original moisture-free and ash-free plant material.

Extractives for lignin determination.—The extractions with 1:2 alcohol-benzene solution, cold water, hot water, and 1-percent boiling hydrochloric acid solution were carried out as already described, except that no corrections were made for the crude protein and the soluble ash in the extract. The total percentage loss due to these extractions was calculated.

Lignin.—This was determined in the extracted plant material by the method of Goss and Phillips (16). The nitrogen in the lignin, determined by the Kjeldahl-Gunning-Arnold (2, p. 25) method, is reported in the tables as percentage of the crude lignin and also as percentage of the original moisture-free and ash-free plant material. The carbohydrates generally associated with lignin in the plant do not interfere with the determination of lignin by this method (39).

Methoxyl in the crude lignin.—Methoxyl was determined in a portion of the crude lignin by the method already referred to. The result was calculated as percentage of the ash-free crude lignin.

Methoxyl in pure lignin.—The percentage of methoxyl in the crude lignin was determined, and the result was calculated on the basis of ash-free and crude protein-free ($N \times 6.25$) lignin. The term “pure lignin,” as used here and in the tables, has been defined in a previous communication (38, p. 307).

RESULTS

The results obtained are recorded in 9 tables and illustrated graphically in 9 figures.⁴ Tables 1, 2, 3, and 4 show the percentage composition of the culms, sheaths, and leaves,⁵ the chaff, the rachises and branches of the panicle, and the roots, respectively, of the oat plant at different stages of growth.

⁴ The data relating to the last three harvest periods of the culms, sheaths, and leaves, shown in the graphs, represent in all cases the mean values of the percentages in the lower, middle, and upper thirds of the plants.

⁵ The culms, sheaths, and leaves were considered as a unit in obtaining the various data recorded in this paper.

TABLE 1.—Composition¹ of culms, sheaths, and leaves of oat plants at different stages of growth

Harvest No.	Age of plants	Ash in original plant material, A	Nitrogen in original plant material		Crude protein (N×6.25)		Methoxyl in original plant material		Methoxyl in extracted plant material ²		Alcohol-benzene extractives		Cold-water extractives		Hot-water extractives		1-percent hydrochloric acid extractives		Total ex-tractives		Uronic acids (as anhy-drides)		Total fur-fural yielded		
			A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
Days	7	Pct.	16.07	5.12	Pct.	0.69	0.81	Pct.	0.14	0.17	Pct.	30.08	35.85	Pct.	10.76	1.24	Pct.	10.98	Pct.	51.33	Pct.	6.98	Pct.	6.50	Pct.
1	14	16.78	5.53	6.64	34.56	41.50	0.80	0.80	0.17	0.20	26.55	32.26	13.72	18.88	1.63	1.96	6.60	7.93	50.80	61.03	7.90	8.32	6.29	7.74	
2	21	17.98	5.60	6.83	35.00	42.69	0.82	0.90	0.23	0.28	21.78	26.57	9.51	11.59	1.59	1.94	10.14	12.36	43.02	52.45	7.00	8.54	7.60	8.54	
3	28	16.18	6.04	7.20	37.75	45.00	0.80	0.95	0.24	0.29	20.64	24.63	7.48	8.93	1.20	1.43	12.54	14.96	41.86	49.95	7.34	7.57	7.97	9.03	
4	35	14.71	5.62	6.59	35.12	41.19	0.74	0.86	0.21	0.25	15.81	18.53	10.05	11.78	1.58	1.85	11.77	13.80	39.21	45.96	6.76	7.62	7.55	8.86	
5	42	15.56	4.19	4.96	26.19	31.00	0.84	0.99	0.24	0.29	20.71	24.53	11.11	13.16	1.56	1.84	13.10	15.31	40.48	55.04	6.64	6.68	8.19	9.69	
6	49	14.25	2.65	3.09	16.56	19.31	1.12	1.30	0.43	0.50	16.32	17.86	12.61	14.71	1.49	1.74	16.99	19.81	46.41	64.12	5.12	5.98	10.65	12.00	
7	56	10.35	2.13	2.38	13.31	14.88	1.56	1.73	0.81	0.90	13.34	14.88	12.92	14.41	1.80	2.01	20.96	23.58	49.02	54.08	4.98	5.56	11.82	13.02	
8	63	13.44	1.13	1.30	7.06	8.12	2.24	2.58	1.43	1.55	12.05	13.08	8.19	9.46	1.13	1.31	23.23	26.83	41.95	48.45	4.38	5.04	14.94	17.45	
9	70	7.91	0.85	0.92	5.31	5.75	2.56	2.78	1.50	1.68	13.02	14.59	8.82	9.68	1.13	1.23	22.44	25.15	43.31	50.78	4.90	5.49	12.92	14.48	
10	77	10.80	0.76	0.85	4.75	5.31	2.73	3.05	1.55	1.75	11.28	12.77	6.94	7.86	1.03	1.15	22.35	24.21	43.21	48.93	5.10	5.90	13.38	15.15	
11	84	11.68	0.57	0.64	3.56	4.00	2.84	3.21	1.55	1.75	11.28	12.77	6.94	7.86	1.03	1.21	22.35	24.21	43.21	48.93	5.10	5.70	14.06	15.70	
12	91	10.49	0.31	0.35	1.94	2.19	3.40	3.80	1.89	2.11	7.48	8.36	8.01	8.88	1.21	1.34	24.43	27.09	40.65	48.86	4.82	5.34	13.98	15.50	
13, lower third	91	9.81	0.40	0.44	2.50	2.75	3.10	3.43	1.81	2.01	6.64	7.36	6.31	7.06	1.03	1.24	25.07	27.99	43.58	48.56	4.76	5.20	14.69	16.44	
13, upper third	91	10.49	0.40	0.44	2.50	2.75	3.10	3.43	1.81	2.01	6.64	7.36	6.31	7.06	1.03	1.24	25.07	27.99	43.58	48.56	4.76	5.20	14.69	16.44	
14, lower third	98	10.67	0.67	0.73	2.69	3.00	2.96	3.31	2.26	2.54	5.85	6.56	6.63	7.44	1.03	1.24	25.07	27.99	43.58	48.56	4.76	5.20	14.69	16.44	
14, middle third	98	10.20	0.33	0.37	2.06	2.31	3.18	3.57	2.06	2.30	1.42	1.59	7.48	8.33	1.27	1.42	26.89	30.03	37.22	42.85	5.10	5.70	14.67	16.52	
14, upper third	98	10.45	0.27	0.30	1.69	1.88	3.60	3.76	2.06	2.30	3.60	4.02	6.60	7.38	1.27	1.42	26.89	30.03	37.22	42.85	5.10	5.70	14.67	16.52	
15, lower third	105	11.08	0.31	0.35	1.94	2.19	3.11	3.47	2.06	2.30	3.60	4.02	6.60	7.38	1.27	1.42	26.89	30.03	37.22	42.85	5.10	5.70	14.67	16.52	
15, middle third	105	10.59	0.30	0.34	1.88	2.12	2.39	2.89	2.12	2.35	2.89	3.25	6.37	7.16	1.35	1.52	28.83	29.05	36.44	40.98	5.94	6.04	14.29	15.98	
15, upper third	105	11.07	0.35	0.39	2.19	2.44	2.98	3.36	2.10	2.35	3.24	3.63	6.24	6.98	1.18	1.32	28.69	29.18	36.75	41.11	5.94	5.36	15.22	17.11	

For footnotes, see end of table.

TABLE 1.—Composition of culms, sheaths, and leaves of oat plants at different stages of growth—Continued ¹

Harvest No.	Age of plants	Pentosans ¹			Cross and Bevan cellulose		Furfural in Cross and Bevan cellulose ⁴	Xylan in Cross and Bevan cellulose			Cellulose			Reducing sugars (as dextrose)			Nonreducing sugars (as sucrose)			Total extractions in sample for lignin determination ⁵			Lignin			Nitrogen in crude lignin			Methoxyl in ash-free lignin ⁶	Ash in lignin ⁷	Methoxyl in lignin ⁸	Methoxyl in "pure" lignin																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
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¹ Results in column A were calculated on oven-dried (105° C.) material; those in column B were calculated on oven-dried (105° C.) and ash-free material.² Results calculated on the original unextracted plant material.³ Corrected for furfural from uronic acids.⁴ Calculated as percentage of ash-free Cross and Bevan cellulose.⁵ Calculated as percentage of original moisture-free plant material.⁶ Calculated as percentage of crude lignin.⁷ Calculated as percentage of original plant material.⁸ Calculated as percentage of ash-free crude lignin.

TABLE 2.—Composition ¹ of chaff of oat plants at different stages of growth

Harvest No.	Age of plants	Ash in original plant material, A	Nitrogen in original plant material		Crude protein (N x 6.25)	Methoxyl in original plant material	Methoxyl in extracted plant material	Alcohol-benzene extractives	Cold-water extractives	Hot-water extractives	1-percent hydrochloric acid extractives	Total extractives	Uronic acids (as anhydrides)	Total furfural yielded
			A	B	A	B	A	B	A	B	A	B	A	B
	Days	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
9	63	6.44	1.88	2.01	11.75	12.56	1.88	2.01	3.67	3.93	1.35	1.44	5.88	5.12
10	70	8.37	1.90	2.14	12.25	13.38	1.83	2.00	7.37	8.04	1.95	2.13	5.40	5.22
11	77	12.17	1.83	2.15	11.73	13.44	1.80	2.05	4.23	4.85	2.30	2.63	4.64	4.26
12	84	16.36	1.82	2.15	8.45	9.94	1.66	1.98	4.96	5.95	3.57	4.20	4.74	4.26
13	91	18.33	1.62	1.22	6.38	7.62	1.63	1.97	5.10	6.16	6.40	7.65	5.24	4.80
14	98	18.13	.77	.94	4.38	5.88	1.43	.97	4.23	5.10	3.68	4.49	4.42	4.05
15	105	18.29	.77	.94	4.81	5.88	1.76	.96	4.82	5.90	4.28	5.24	4.24	3.14

Harvest No.	Age of plants	Pentosans ²		Cross and Bevan cellulose		Xylan in Cross and Bevan cellulose		Furfural from Cross and Bevan cellulose ⁴		Reducing sugars (as dextrose)		Nonreducing sugars (as sucrose)		Total extractives in sample for lignin determination ⁵	Lignin	Nitrogen in crude lignin	Methoxyl in "pure" lignin	Ash in lignin ⁶
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	A	A
	Days	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
9	63	31.74	33.93	33.7	36.1	16.67	25.86	8.71	9.34	1.48	1.58	1.40	1.60	58.93	6.58	2.74	9.54	19.95
10	70	25.91	28.27	29.7	32.4	15.85	24.60	7.31	7.97	1.70	1.59	1.63	1.19	58.67	7.08	2.57	10.00	22.47
11	77	24.23	27.80	29.6	33.9	17.53	27.20	8.05	9.22	2.42	2.77	1.68	2.21	57.99	6.32	2.50	9.25	40.89
12	84	23.48	27.65	26.7	31.5	16.38	25.44	6.79	8.01	2.20	2.59	1.75	1.88	57.72	6.70	1.65	10.26	46.96
13	91	23.68	26.32	28.3	33.8	16.73	25.93	7.34	8.75	.96	1.53	1.70	.96	56.06	6.75	1.41	10.26	47.10
14	98	25.80	31.51	29.7	36.2	15.04	23.31	6.92	8.44	.63	.79	.26	.31	55.63	7.10	.83	10.78	52.21
15	105	26.03	31.86	29.3	35.8	15.34	23.79	6.97	8.52	.54	.69	.23	.22	56.53	7.23	1.15	9.97	51.05

¹ Results in column A were calculated on oven-dried (105° C.) material; those in column² B were calculated on oven-dried (105° C.) and ash-free material.³ Calculated on the original unextracted plant material.⁴ Corrected for furfural from uronic acids.⁵ Calculated as percentage of ash-free Cross and Bevan cellulose.⁶ Calculated as percentage of original moisture-free plant material.⁷ Calculated as percentage of crude lignin.⁸ Calculated as percentage of original plant material.⁹ Calculated as percentage of ash-free crude lignin.

TABLE 3.—Composition ¹ of the rachises and branches of the panicles of oat plants at different stages of growth

Harvest No.	Age of plants	Ash in original plant material, A		Nitrogen in original plant material		Crude protein (N X 6.25)		Methoxyl in original plant material		Methoxyl in extracted plant material ²		Alcohol-benzene extractives		Cold-water extractives		Hot-water extractives		1-percent hydrochloric acid extractives		Total extractives		Uronic acids (as anhydrides)		Total fufural yielded	
		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
9.....	Days 63	4.83	1.65	1.73	10.31	2.22	2.33	1.23	1.29	6.39	6.71	4.93	5.17	2.24	2.35	33.48	35.18	33.20	31.74	47.04	49.41	5.46	5.74	19.49	20.48
10.....	70	4.86	1.38	1.45	8.62	9.06	2.41	2.54	1.41	1.48	11.70	12.30	3.28	3.44	2.00	2.10	30.20	31.74	47.18	49.58	4.56	4.80	17.91	18.83	
11.....	77	5.95	1.38	1.47	8.62	9.19	2.41	2.56	1.39	1.47	10.16	10.80	3.73	3.96	2.69	2.86	33.26	34.86	47.86	50.88	4.36	4.60	16.00	17.02	
12.....	84	7.32	1.12	1.21	7.00	7.56	2.55	2.76	1.44	1.56	11.39	12.29	3.35	3.31	3.04	3.10	33.35	34.90	48.90	52.77	4.08	4.40	15.46	16.68	
13.....	91	10.67	.70	.78	4.38	4.88	2.64	2.95	1.42	1.59	9.79	10.96	5.77	6.46	1.83	2.04	30.56	32.41	47.95	53.67	4.34	4.86	17.06	19.10	
14.....	98	8.75	.62	.68	3.88	4.25	2.86	3.13	1.56	1.71	8.57	9.39	3.58	3.92	1.73	2.33	31.45	34.47	45.73	50.11	4.30	4.70	17.74	19.44	
15.....	105	9.63	.58	.64	3.62	4.00	2.90	3.20	1.55	1.72	10.86	12.02	4.18	4.63	1.73	1.91	25.62	27.78	46.39	51.34	4.32	4.76	17.88	19.78	

Harvest No.	Age of plants	Pentosans ³		Cross and Bevan cellulose		Furfural from Cross and Bevan cellulose ⁴		Xylan in Cross and Bevan cellulose		Cellulose		Reducing sugars (as dextrose)		Nonreducing sugars (as sucrose)		Total extractives in sample for lignin determination ⁵		Lignin		Nitrogen in crude lignin ⁶		Methoxyl in ash-free lignin ⁷		Ash in lignin ⁸		Methoxyl in "pure" lignin	
		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.		Ped.	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
9.....	Days 63	31.29	32.88	36.4	38.3	15.97	24.79	9.02	9.49	27.4	28.8	1.08	1.13	2.20	2.30	56.22	7.62	8.01	2.42	0.24	0.25	11.18	7.51	13.37	13.37		
10.....	70	28.93	30.42	36.9	38.8	16.08	24.95	9.21	9.68	27.7	29.1	1.21	1.27	5.27	5.53	54.78	8.04	8.45	2.15	.22	.23	12.72	8.59	14.90	14.90		
11.....	77	25.73	27.39	33.7	35.8	17.19	26.69	8.99	9.56	24.7	26.3	1.78	1.89	2.81	2.99	55.95	7.84	8.34	1.95	.26	.28	12.21	13.08	14.73	14.73		
12.....	84	24.91	26.88	33.6	36.2	16.83	26.14	8.78	9.46	24.8	26.8	2.37	2.55	1.79	1.92	56.36	8.29	8.94	1.95	.22	.24	12.27	14.19	14.30	14.30		
13.....	91	27.56	30.85	35.5	39.8	15.61	24.25	8.61	9.65	26.9	30.2	.82	.91	1.81	2.02	55.96	8.01	8.97	1.20	.13	.14	14.10	17.07	15.50	15.50		
14.....	98	28.74	31.50	41.9	45.9	16.44	25.49	10.68	11.70	31.2	34.2	---	---	---	---	52.21	8.47	9.28	.95	.10	.11	15.20	12.96	16.30	16.30		
15.....	105	28.97	32.02	41.8	46.3	16.28	25.27	10.56	11.70	31.3	34.6	---	---	---	---	52.47	8.44	9.34	.83	.09	.10	14.66	15.53	15.62	15.62		

¹ Results in column A were calculated on oven-dried (105° C.) material; those in column B were calculated on oven-dried (105° C.) and ash-free material.² Results calculated on the original unextracted plant material.³ Corrected for fufural from uronic acids.⁴ Calculated as percentage of ash-free Cross and Bevan cellulose.⁵ Calculated as percentage of original moisture-free plant material.⁶ Calculated as percentage of crude lignin.⁷ Calculated as percentage of original plant material.⁸ Calculated as percentage of ash-free crude lignin.

TABLE 4.—Composition¹ of roots of oat plants at different stages of growth

Harvest No.	Age of plants	Ash in original plant material, %	Nitrogen in original plant material		Crude protein (N×6.25)		Methoxyl in original plant material		Methoxyl in extracted plant material ²		Alcohol-benzene extractives		Cold-water extractives		Hot-water extractives		1-percent hydrochloric acid extractives		Total extractives		Uronic acids (as anhydrides)	
			A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Days		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
1	7	11.55	1.94	2.19	12.12	13.69	1.48	1.67	0.78	0.88	9.90	11.26	2.24	2.53	2.91	3.29	23.49	33.34	44.60	50.42	5.98	6.40
2	14	10.99	2.38	2.67	14.88	16.68	2.11	2.37	.82	.92	9.71	10.91	5.95	6.68	2.75	3.09	26.40	29.66	44.81	50.34	5.88	7.00
3	21	15.58	1.84	2.18	11.50	13.62	1.47	1.74	.82	.97	6.12	7.24	2.76	3.57	2.83	3.33	28.98	34.29	42.66	48.15	5.83	6.40
4	28	12.29	2.00	2.28	12.50	14.25	1.54	1.76	.81	.92	7.17	8.17	5.49	6.22	2.83	3.33	23.98	30.70	42.79	48.33	5.81	6.41
5	35	12.01	1.25	1.42	7.81	8.88	1.83	2.07	1.02	1.15	4.06	4.61	4.39	4.99	2.33	2.63	23.99	34.08	40.15	45.36	5.06	5.76
6	42	13.08	.95	1.09	5.94	6.81	2.19	2.51	1.26	1.45	2.91	3.35	3.07	3.53	2.33	2.22	30.24	34.73	38.15	43.39	5.04	5.80
7	49	20.69	.64	.81	4.00	5.09	2.31	2.90	1.44	1.69	1.93	2.43	6.88	8.68	2.39	3.01	24.00	30.25	35.30	44.37	4.60	5.80
8	55	14.73	.50	.51	3.12	3.50	2.75	3.22	1.61	1.83	6.92	8.12	5.55	6.51	2.22	2.61	24.94	29.25	39.63	46.49	4.64	5.44
9	63	11.35	.42	.49	3.22	3.61	3.20	3.61	1.92	2.16	3.95	4.46	4.82	5.43	2.03	2.34	23.56	32.21	39.41	44.44	5.06	5.76
10	70	14.78	.37	.37	2.62	3.05	3.22	3.77	1.93	2.26	3.01	3.88	4.49	5.23	2.06	2.42	26.74	31.37	38.24	44.90	4.40	5.18
11	77	10.72	.32	.35	2.03	2.31	3.57	4.00	2.05	2.29	5.22	5.84	2.13	2.38	2.03	2.30	25.84	32.30	38.34	42.82	4.84	5.40
12	84	8.96	.30	.32	1.83	2.09	3.80	4.17	2.30	2.52	1.01	1.11	2.70	3.03	2.04	2.46	31.26	34.23	37.77	40.93	4.72	5.26
13	91	5.78	.27	.30	1.69	1.88	4.06	4.31	2.50	2.63	6.30	6.94	2.68	2.84	2.10	2.23	30.84	32.74	33.74	42.18	4.78	5.08
14	98	9.17					4.82	5.30	2.37	2.62	6.30	6.94	1.20	1.33	1.03	1.13	30.00	33.03	38.53	42.43	4.86	5.34
15	105																					

For footnotes, see end of table.

TABLE 4.—Composition of roots of oat plants at different stages of growth—Continued

Harvest No.	Age of plants	Total furfural yielded		Pentosans ³		Cross and Bevan cellulose		Furfural from Cross and Bevan cellulose ³	Xylan in Cross and Bevan cellulose		Cellulose		Total extractives in sample for lignin determination ⁶	Lignin		Nitrogen in crude lignin				Methoxyl in ash-free lignin ⁹	Ash in lignin ⁷	Methoxyl in "pure" lignin		
		Total furfural yielded		Pentosans ³		Cross and Bevan cellulose			Xylan in Cross and Bevan cellulose		Cellulose			Lignin		Nitrogen in crude lignin								
		A	B	A	B	A	B		A	B	A	B		A	B	A	B	A	B				A	B
Days ⁷		Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.		
1	14	15.12	16.99	23.75	26.65	40.2	47.6	13.52	21.40	8.60	10.19	31.6	37.4	5.11	5.74	2.83	30	34	32.72	34.17	12.09			
2	21	15.10	17.90	23.63	23.01	40.2	47.6	13.52	21.40	8.60	10.19	31.6	37.4	5.11	5.74	2.83	30	34	32.72	34.17	12.09			
3	28	15.49	17.67	24.40	27.84	39.1	44.6	13.73	25.80	8.33	9.50	30.9	35.2	5.18	6.14	1.57	24	29	56.22	56.22	11.29			
4	35	17.73	20.15	23.44	32.32	46.4	52.8	16.70	25.80	12.01	13.67	34.4	39.1	5.28	6.02	2.21	30	34	47.67	47.67	11.32			
5	42	17.73	20.15	23.44	32.32	46.4	52.8	16.70	25.80	12.01	13.67	34.4	39.1	5.28	6.02	2.21	30	34	46.95	46.95	14.76			
6	49	16.52	19.00	26.35	30.33	42.4	48.8	13.98	22.38	10.20	11.85	37.0	42.3	6.40	7.79	1.96	29	29	46.95	46.95	15.70			
7	56	14.62	18.43	23.29	29.36	40.6	51.1	14.39	22.38	10.20	11.85	37.0	42.3	6.40	7.79	1.96	29	29	46.95	46.95	15.70			
8	63	13.59	15.95	21.53	25.26	39.0	45.8	13.45	20.83	9.05	11.40	31.6	37.1	6.43	8.41	1.76	21	22	46.95	46.95	16.40			
9	70	13.12	17.05	23.97	27.05	43.0	48.5	13.39	20.83	8.31	9.75	31.6	37.1	6.43	8.41	1.76	21	22	46.95	46.95	16.40			
10	77	14.32	16.81	22.84	26.81	42.4	49.8	13.36	20.83	8.31	9.75	31.6	37.1	6.43	8.41	1.76	21	22	46.95	46.95	16.40			
11	84	13.60	17.47	24.83	27.87	43.9	51.4	14.00	20.89	10.11	11.88	32.3	37.9	6.30	8.99	1.95	17	20	46.95	46.95	17.05			
12	91	16.43	18.07	26.37	28.97	47.6	52.6	13.06	20.89	10.11	11.88	32.3	37.9	6.30	8.99	1.95	17	20	46.95	46.95	17.05			
13	98	16.88	17.60	26.37	28.97	47.6	52.6	13.41	20.89	10.11	11.88	32.3	37.9	6.30	8.99	1.95	17	20	46.95	46.95	17.05			
14	105	16.10	17.73	25.72	23.33	46.0	50.7	13.50	23.90	11.39	12.20	34.9	38.5	11.07	12.54	.47	.08	.09	30.26	30.26	17.44			
15									24.07	11.07	12.20	34.9	38.5	11.39	12.54	.47	.08	.09	30.85	30.85	18.19			

¹ Results in column A were calculated on oven-dried (105° C.) material; those in column B were calculated on oven-dried (105° C.) and ash-free material.² Results calculated on the original unextracted plant material.³ Corrected for furfural from uronic acids.⁴ Samples from harvests 1 and 2 were combined and analyzed.⁵ Calculated as percentage of ash-free Cross and Bevan cellulose.⁶ Calculated as percentage of original moisture-free plant material.⁷ Calculated as percentage of crude lignin.⁸ Calculated as percentage of original plant material.⁹ Calculated as percentage of ash-free crude lignin.

ASH

The percentage of ash in the culms, sheaths, and leaves (table 1 and fig. 1) at first increased, reaching a maximum of 17.98 percent when the plants were 21 days old. Subsequently, there was a general decrease, although a rather irregular one, as the plants became older. This is in agreement with the results obtained by Norton (32) and by Fagan and Watkins (13). Similar results were obtained with wheat plants by Lawes and Gilbert (24), Phillips, Davidson, and Weihe (36), and Malhotra (26, 27). Norman (28) and Phillips and Goss (38) obtained similar results with barley plants. The percentage of ash in the chaff (table 2 and fig. 1) increased regularly as the plants matured.

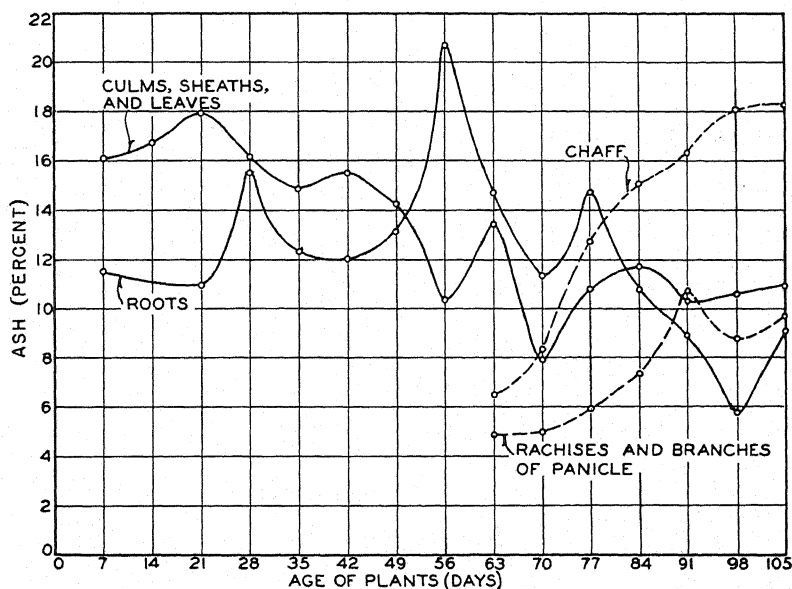


FIGURE 1.—Percentage of ash in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth. (Ash values are on a moisture-free basis.)

When the plants were 63 days old it was only 6.44, whereas at maturity it had increased to 18.29 percent. The percentage of ash in the rachises and branches of the panicles (table 3 and fig. 1) increased regularly until the plants were 91 days old, after which it decreased somewhat. The variations in the percentages of ash in the roots (table 4 and fig. 1) at different stages of the development of the plants were rather irregular. In spite of the fact that the roots were washed repeatedly with water, it was not possible to free them entirely from particles of sand and other inorganic matter. Accordingly the figures on the percentage of ash in the roots are presented with some reserve.

NITROGEN AND CRUDE PROTEIN

After an early increase, the percentage of nitrogen and crude protein in the culms, sheaths, and leaves decreased generally as the plants matured (table 1 and fig. 2). The data obtained by Shaw and Wright (46) in their studies on the percentage of nitrogen in the corn plant at different stages of growth showed a similar tendency. Results of like character were obtained with wheat plants by Phillips, Davidson, and Weihe (36), and with barley plants by Phillips and Goss (38). In the plants from the last three harvest periods there were some differences, although not very significant ones, in the percentages of nitrogen and crude protein in the culms from the top downward. In the culms from harvests 13 (91 days) and 15 (105

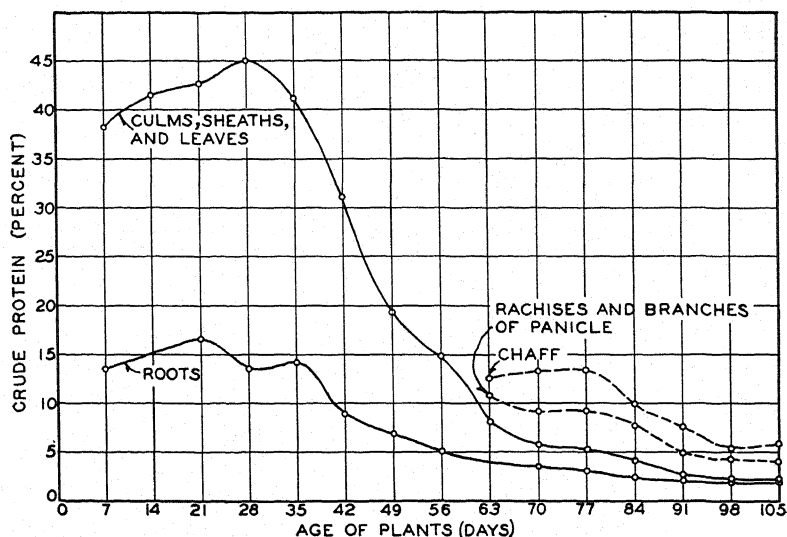


FIGURE 2.—Percentage of crude protein (on moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

days) the percentages of nitrogen and crude protein increased from the bottom of the stalk upward. However, this was not true of the culms from harvest 14 (98 days). The nitrogen and crude protein in the chaff and in the rachises and branches of the panicles (tables 2 and 3 and fig. 2) decreased generally, although not consistently, as the plants matured, but were considerably higher than in the culms, sheaths, and leaves from plants at the same stage of development. Thus at maturity the average percentage of crude protein (calculated on a moisture-free and ash-free basis) in the culms, sheaths, and leaves amounted to 2.10, whereas in the chaff and in the rachises and branches of the panicles the percentages of crude protein, calculated on the same basis, were 5.88 and 4.00, respectively. With the exception of the early stages of growth, the percentages of nitrogen and crude protein in the roots decreased regularly and consistently as the plants matured and became senescent (table 4 and fig. 2).

METHOXYL IN ORIGINAL PLANT MATERIAL

The percentages of methoxyl in the original plant material represent the sum of the percentages of methoxyl present either in the form of methyl esters, as in the pectins, or as methyl ethers, as in lignin. In the culms, sheaths, and leaves (table 1 and fig. 3, A) the percentage of

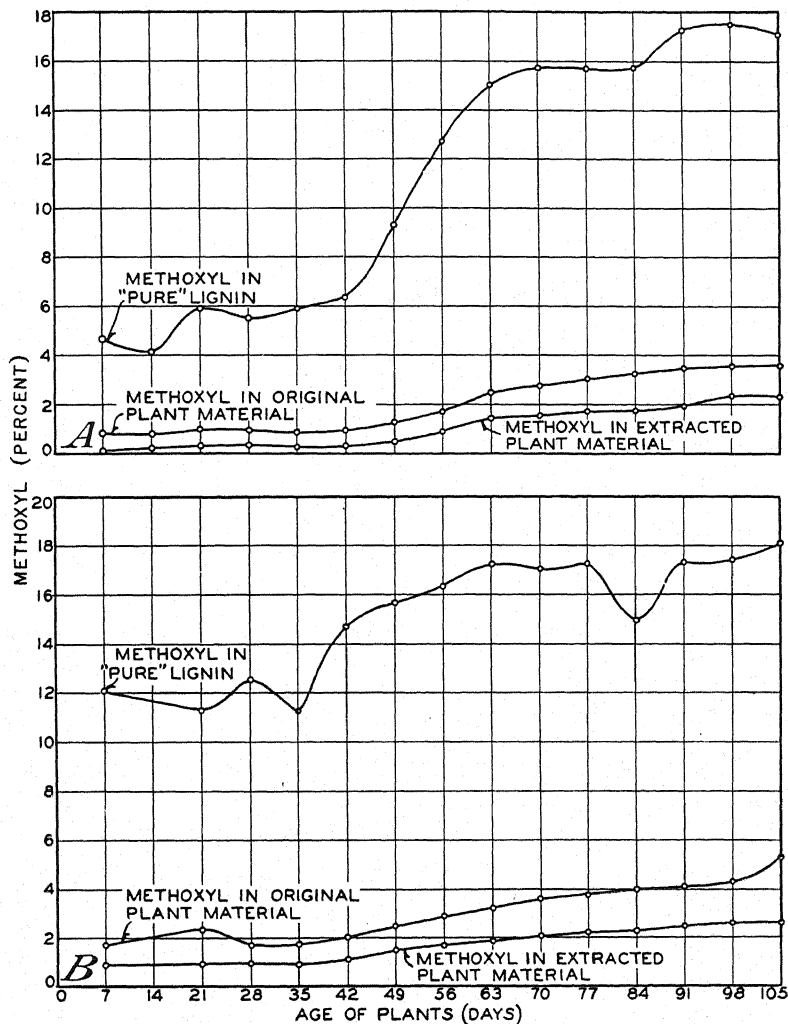


FIGURE 3.—Percentage of methoxyl in original plant material, in extracted plant material, and in "pure" lignin, in (A) culms, sheaths, and leaves, and (B) roots of oat plants at successive stages of growth. (All values are on a moisture-free and ash-free basis.)

methoxyl increased, in general, with the increase in the age of the plants until the plants were 91 days old. Subsequently, although there were some variations, the percentages on the whole were of the same general order of magnitude. The percentage of methoxyl in

the chaff (table 2) showed no appreciable change, but in the rachises and branches of the panicles (table 3) it increased regularly and consistently with the increase in the age of the plants. The percentage of methoxyl in the roots (table 4 and fig. 3, *B*) increased regularly with the increase in the age of the plants, except in the sample from the fourth harvest (28 days). The percentage of methoxyl in the roots was greater than that in any of the other parts of the oat plant. Thus at maturity, the percentage of methoxyl in the roots calculated on a moisture-free and ash-free basis was 5.30, while in the combined culms, sheaths, and leaves, in the chaff, and in the combined rachises and branches of the panicles the percentages calculated on the same basis were 3.55, 2.16, and 3.20, respectively.

METHOXYL IN EXTRACTED PLANT MATERIAL

The figures on methoxyl in the extracted plant material represent essentially methoxyl groups present as methyl ethers. The chief source, although not the only one, for these firmly bound methoxyl groups is lignin. In the culms, sheaths, and leaves (table 1 and fig. 3, *A*), the percentage of firmly bound methoxyl, calculated on the basis of the original unextracted plant materials, increased, in general, with the increase in the age of the plants. In the samples from the last three harvest periods, the percentages of methoxyl in the culms decreased generally from the bottom of the stalks upward. The percentage of firmly bound methoxyl groups in the chaff (table 2) increased slightly, and in the rachises and branches of the panicles (table 3) it increased somewhat more as the plants matured. The percentages of methoxyl in both cases, however, were lower than those in the culms, sheaths, and leaves from plants at the same stage of development. The percentage of firmly bound methoxyl in the roots (table 4 and fig. 3, *B*) increased generally with the increase in the age of the plants. The percentages were, in the main, higher than those in the culms, sheaths and leaves, chaff, and rachises and branches of the panicles taken from plants at the same stage of development. Attention is called to the fact that the percentage of lignin was higher in the roots than in other parts of the plant. This would explain the higher percentage of firmly bound methoxyl found in the roots, as an increase in the percentage of lignin would naturally cause a corresponding increase in the percentage of methoxyl in the extracted plant material.

ALCOHOL-BENZENE EXTRACTIVES

The percentage of alcohol-benzene extractives in the culms, sheaths, and leaves (table 1) decreased as the plants grew older, but the decrease was not uniform. As was pointed out in a previous communication (38, p. 309), an alcohol-benzene solution is not a selective solvent, and it removes a heterogeneous class of compounds, such as fatty and waxy substances, volatile oils, pigments, and various resinous complexes. This explains, at least in part, the irregular results obtained. The variations in the percentages of alcohol-benzene extractives in the chaff (table 2), in the rachises and branches of the panicles (table 3), and in the roots (table 4) were also irregular.

COLD-WATER EXTRACTIVES

After an increase in the percentage of cold-water extractives in the culms, sheaths, and leaves (table 1) from the second harvest period, the percentage decreased, although irregularly, as the plants matured. In the samples from harvests 12 to 15 the percentages ranged from about 7 to 9. In the chaff (table 2), rachises and branches of the panicles (table 3), and the roots (table 4) the percentages were irregular, and no definite conclusions can be drawn from them. The procedure used in this investigation for determining cold-water extractives gives results that represent the sum of the various substances (except water-soluble nitrogenous complexes and ash) removed by this treatment. It is not at all surprising, therefore, that the percentages do not show a uniform and regular trend.

HOT-WATER EXTRACTIVES

The percentage of hot-water extractives in the culms, sheaths, and leaves (table 1) showed but little variation during the development of the plants. The values for hot-water extractives were in every case considerably lower than those for the cold-water extractives. The values for the hot-water extractives in the rachises and branches of the panicles (table 3) showed the same tendency as those in the culms, sheaths, and leaves. In the chaff (table 2), however, the percentage of hot-water extractives increased with the increase in the age of the plants until the plants were 91 days old, after which it declined somewhat. The percentage of hot-water extractives in the roots (table 4) declined, although not regularly, as the plants grew older and matured.

ONE PERCENT HYDROCHLORIC ACID EXTRACTIVES

The percentage of 1 percent hydrochloric acid extractives in the culms, sheaths, and leaves (table 1) increased, although not in a uniform manner, as the plants grew older. The maximum percentage was reached when the plants were 98 days old. This general increase in the 1 percent hydrochloric acid extractives may be attributed to the fact that certain of the reserve carbohydrates, for example the hemicelluloses, in the culms, sheaths, and leaves generally increase as the plant grows older. In the chaff (table 2) and in the rachises and branches of the panicle (table 3) (calculated on a moisture-free and ash-free basis), there were no appreciable variations in the percentage as the plants developed and matured.

TOTAL EXTRACTIVES

The figures on total extractives represent the sum of the percentages of alcohol-benzene, cold-water, hot-water, and 1 percent hydrochloric acid extractives. In the culms, sheaths, and leaves (table 1) the percentage generally declined, although not regularly, as the plants matured. When the plants were young, approximately 61 percent of the plant substance (calculated on a moisture-free and ash-free basis) could be removed by successive extraction with alcohol-benzene solution, cold water, hot water, and 1 percent hydrochloric acid, while at maturity only about 41 percent could thus be extracted. In the roots (table 4)

the percentages of total extractives showed the same trend as that of the culms, sheaths, and leaves. However, the percentages in the chaff (table 2) and in the rachises and branches of the panicles (table 3) did not vary appreciably with the increase in the age of the plants.

The results on the total extractives of the samples used for determination of lignin, which were not corrected for the crude protein and soluble ash, showed the same trend as those discussed above.

URONIC ACIDS

Uronic acids are constituents of two substances of vegetable origin, namely, the pectins and the hemicelluloses. The uronic acid content of these two materials, however, is not the same; the pectins contain a much greater percentage of these acids than do the hemicelluloses.

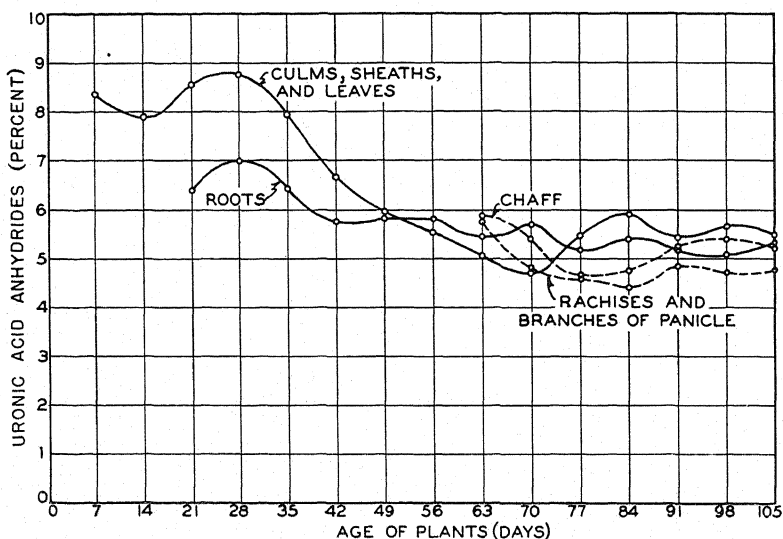


FIGURE 4.—Percentage of uronic acid anhydrides (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

As the plants grow older, the percentage of pectin, particularly of lignified tissues, decreases, so that the percentage of uronic acids in the plant would necessarily decrease. This explains the results found in the determination of the percentage of uronic acids (calculated as anhydrides) in the culms, sheaths, and leaves (table 1 and fig. 4) of the oat plants. Similar results were obtained by Phillips and Goss (38) in their experiments with barley. The percentage of uronic acids (calculated as anhydrides) in the chaff (table 2 and fig. 4) and in the rachises and branches of the panicles (table 3 and fig. 4) showed the same tendency, although the decrease in percentage was not uniform and regular. The percentage of uronic acids (calculated as anhydrides) in the roots (table 4 and fig. 4) had increased slightly in the fourth harvest period (28 days), after which it decreased slowly as the plants developed and matured. In general, the percentage of uronic acids in the culms, sheaths, and leaves was greater than in the

roots taken from plants of the same age and at the same stage of development.

TOTAL FURFURAL

When a plant material is distilled with 12 percent hydrochloric acid, the total furfural obtained is derived from three principal sources, namely, (1) from the uronic acids, (2) from the pentose or pentosan fraction of the polyuronides, and (3) from the pentosan fraction forming an integral part of the Cross and Bevan cellulose. Inasmuch as some of these components increase while others decrease as the plant develops and matures and since the percentages of furfural afforded by these constituents are not the same, the percentages of total furfural recorded in the tables represent an additive effect of several factors of unequal magnitude.

The percentage of total furfural in the culms, sheaths, and leaves (table 1 and fig. 9, A) increased, in general, and reached a maximum

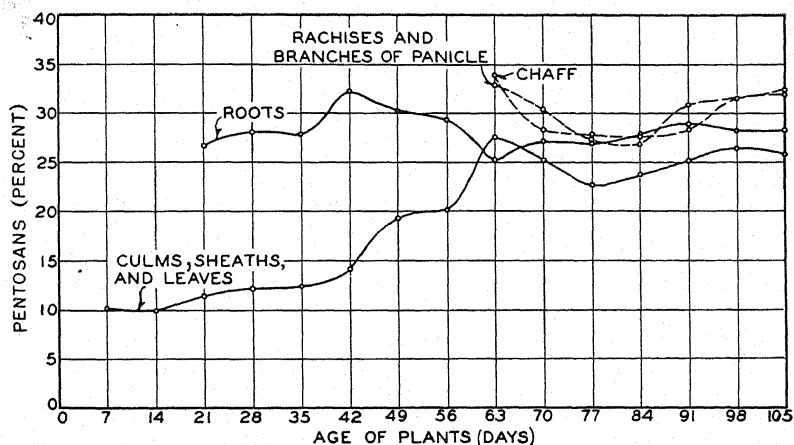


FIGURE 5.—Percentage of pentosans (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicle, and roots of oat plants at successive stages of growth.

when the plants were 63 days old. Subsequently there was a decrease, but at or near maturity the percentage increased again. In the chaff (table 2) and in the rachises and branches of the panicles (table 3), the percentages of total furfural showed no appreciable variation (except in the samples from the ninth harvest) with the increase in the age of the plants. In the roots (table 4 and fig. 9, B), after an increase in the earlier stages of development of the plants, there was no great variation in the percentage of total furfural.

PENTOSANS

The percentage of pentosans was obtained by deducting from the percentage of total furfural the percentage of furfural derived from the uronic acids and calculating the difference as pentosans. These figures represent, therefore, the total pentose or pentosan units in the plant material, irrespective of whether these units occur in the gums, hemicelluloses, pectins, or in the Cross and Bevan cellulose.

Table 1 and figure 5 show that the percentage of pentosans in the culms, sheaths, and leaves increased steadily until the plants were 63 days old. Subsequently there was a small decrease in the percentage, but at maturity it had increased again. With the exception of the lower third of the sample from the thirteenth harvest (91 days), the percentages of pentosans in the samples from the last three harvests increased from the bottom of the stalk upward. In the chaff (table 2 and fig. 5) and in the rachises and branches of the panicles (table 3 and fig. 5), the highest percentage of pentosans was found in the samples from the ninth harvest (63 days). In the plants from subsequent harvests, the percentage of pentosans had decreased, but at maturity it had increased somewhat. In the roots (table 4

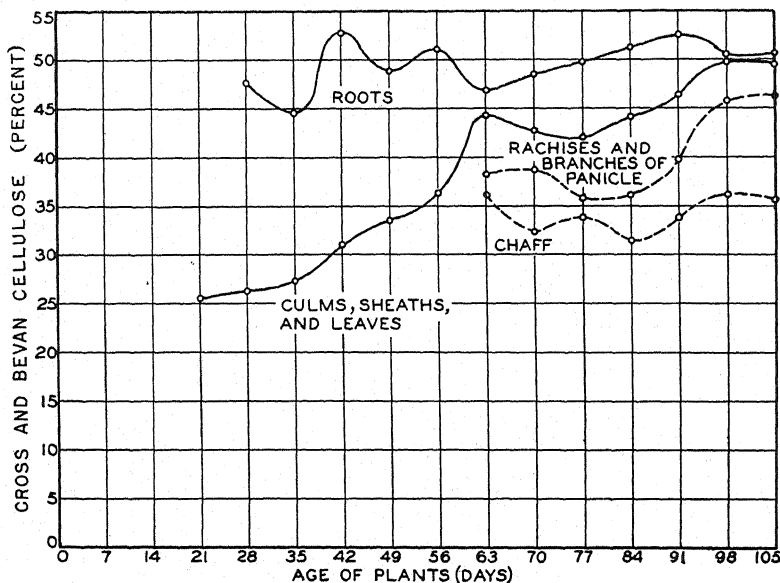


FIGURE 6.—Percentage of Cross and Bevan cellulose (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

and fig. 5), the percentage of pentosans increased in the early stages of the development of the plants, then it decreased somewhat, and in samples taken after the ninth harvest (63 days) there was little variation.

CROSS AND BEVAN CELLULOSE

Table 1 and figure 6 show that the percentage of Cross and Bevan cellulose in the culms, sheaths, and leaves increased from 25.5 percent (calculated on a moisture-free and ash-free basis) in the young seedlings to nearly twice this amount in the mature plants. The increase, however, was not even or regular. Similar results were obtained by Norman (28) and by Phillips and Goss (38) in their experiments with barley. In the main, in the samples from the last three harvest periods, the percentage of Cross and Bevan cellulose decreased in the culms from the bottom upward, although in some cases the decreases

were not significant. The percentage of Cross and Bevan cellulose in the chaff (table 2 and fig. 6) was irregular. From an initial percentage of 36.1 (calculated on moisture-free and ash-free basis), it had decreased to 31.5 when the plants were 84 days old, and then at maturity it had increased to about 36 again. In general, the percentage of Cross and Bevan cellulose in the chaff was found to be considerably less than in the culms, sheaths, and leaves taken from plants of the same age and at the same stage of development. The percentage of Cross and Bevan cellulose in the rachises and branches of the panicles (table 3 and fig. 6) decreased from 38.3 (calculated on a moisture-free and ash-free basis) when the plants were 63 days old to 35.8 when the plants were 77 days old. Subsequently the percentage gradually increased until at maturity it was 46.3. The percentage of Cross and Bevan cellulose in the roots (table 4 and fig. 6) increased very little as the plants grew and developed. Attention is called particularly to the fact that in the young plants the percentage of Cross and Bevan cellulose was much greater in the roots than in the culms, sheaths, and leaves. This has also been found to be true of the percentages of methoxyl in extracted plant material and lignin. The significance of this, insofar as it may throw light on the possible origin of lignin in the plant, will be discussed elsewhere in this paper.

FURFURAL AND XYLAN IN CROSS AND BEVAN CELLULOSE

The percentage of furfural yielded by the Cross and Bevan cellulose of the culms, sheaths, and leaves (table 1 and fig. 9) and, of course, also the xylan (calculated as percentage of the Cross and Bevan cellulose and as percentage of the original plant material) increased somewhat as the plants grew older. The increase, however, was neither consistent nor even. Similar results were obtained by Norman (28) and by Phillips and Goss (38) in their studies of the barley plant and by Norman (29) and Norman and Richardson (30) in their experiments on ryegrass. In the chaff (table 2) and in the rachises and branches of the panicles (table 3) the percentages of furfural (also calculated as xylan) in the Cross and Bevan cellulose did not show any pronounced variation with the increase in the age of the plants. In the roots the variations in percentage were rather irregular (table 4). In the early stages of the development of the plants, the percentage increased, then it decreased, but as the plants approached maturity there was again a small increase.

CELLULOSE

The percentage of cellulose in the culms, sheaths, and leaves (table 1 and fig. 7) increased rapidly as the plants grew older. The increase, however, was not regular. Similar results were obtained by Phillips and Goss (38) in their study of the composition of the barley plant at successive stages of growth. The percentage of cellulose in the chaff (table 2 and fig. 7) did not vary greatly with the increase in the age of the plants. From an initial percentage of 26.8 (calculated on moisture-free and ash-free basis) when the plants were 63 days old, it had decreased to 23.5 when the plants were 84 days old, and then at maturity it had increased again to 27.3. The percentage of cellulose in the rachises and branches of the panicles (table 3 and fig. 7) increased, although not in a regular manner, as the plants grew older.

In the roots, however, the percentage of cellulose did not materially change (table 4 and fig. 7).

REDUCING AND NONREDUCING SUGARS

The percentages of reducing and nonreducing sugars (calculated as glucose and sucrose, respectively) in the culms, sheaths, and leaves (table 1) increased, in general, until heading time (ninth harvest), after which they decreased rapidly so that at maturity the percentages of both were quite negligible. Similar results were reported by Collins and Spiller (7). In the chaff (table 2) and in the rachises and branches of the panicles (table 3), the percentages of reducing and nonreducing sugars showed the same trend as those in the culms, sheaths, and leaves; that is, they increased in the earlier stages of development of the plants, after which they declined rapidly. Be-

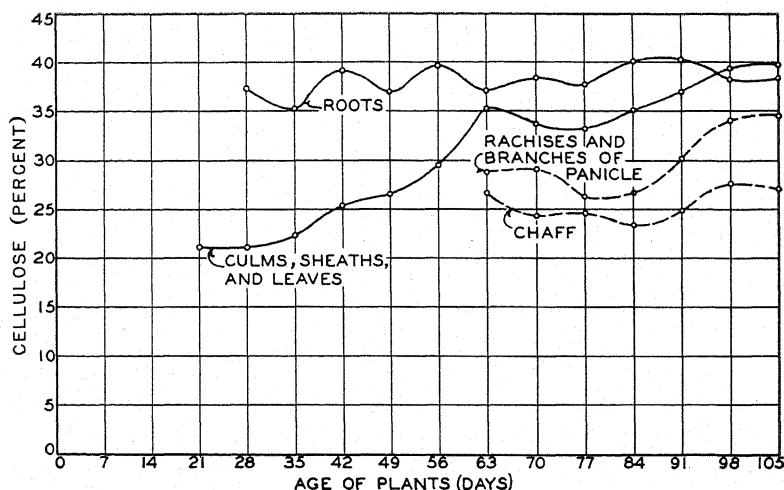


FIGURE 7.—Percentage of cellulose (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

cause of lack of sufficient material, the percentages of reducing and nonreducing sugars in the roots were not determined.

LIGNIN, AND NITROGEN, ASH, AND METHOXYL IN THE LIGNIN

Table 1 and figure 8 show that, in general, the percentage of lignin in the culms, sheaths, and leaves increased as the plants grew older. In the first sample the percentage of lignin, calculated on the moisture-free and ash-free plant material, was 1.86, and in the last sample, when the plants were mature, it was 10.93 (mean value of the percentages of the plants from the last harvest period). Phillips, Davidson, and Weihe (36) found that in the wheat plant the percentage of lignin increased with the age of the plant. Similar results were obtained by Phillips and Goss (38) with the barley plant.

The percentage of nitrogen in the lignin (table 1) decreased as the plant grew and matured. The percentage of nitrogen in the lignin residue from the first sample was 4.34, while in the lignin from the

fully mature plant it was only 0.69 (the means value of the percentages of nitrogen in lignin from the last harvest period). Undoubtedly this was due to the fact that the percentage of nitrogen decreases as the plant grows older and matures.

The figures on the percentage of methoxyl in the ash-free lignin and in the so-called pure lignin from the culms, sheaths, and leaves (table 1 and fig. 3) show that there was a gradual increase as the plants grew older. It is rather difficult to explain this fact on the basis of Klason's (20) hypothesis that lignin is synthesized by the plant from coniferyl alcohol or coniferyl aldehyde. If this were the case the percentage of methoxyl in the lignin would show no such variation.

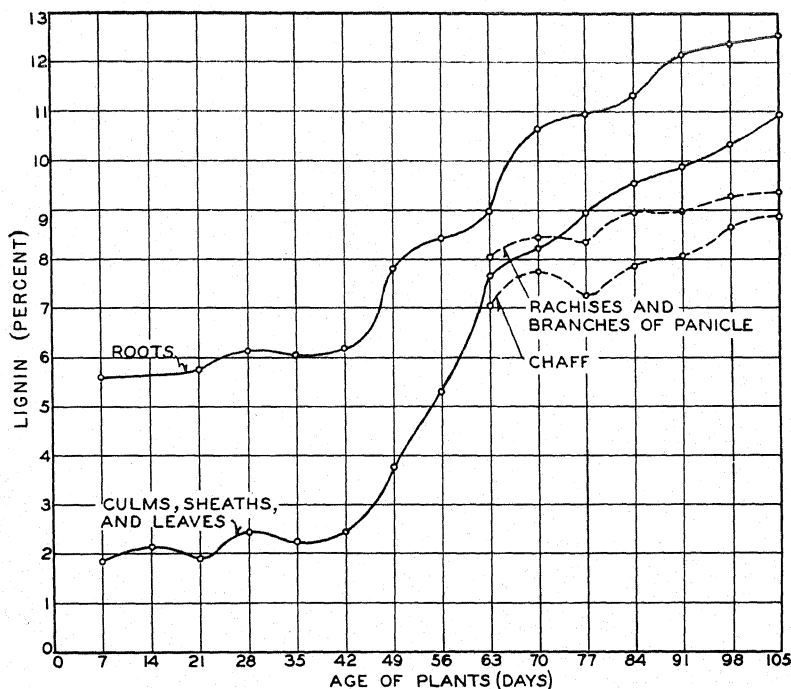


FIGURE 8.—Percentage of lignin (on a moisture-free and ash-free basis) in culms, sheaths, and leaves, chaff, rachises and branches of the panicles, and roots of oat plants at successive stages of growth.

The percentage of ash in the lignin from the culms, sheaths, and leaves (table 1) varied considerably, but in general it decreased as the plants grew older.

The percentage of lignin and the percentages of nitrogen and methoxyl in the lignin in the chaff and in the rachises and branches of the panicles are given in tables 2 and 3, respectively. The percentages of lignin are shown graphically in figure 8. In general, the percentages show the same trend as those of the culms, sheaths, and leaves. The percentages of ash in the lignin, however, differ markedly from those recorded in table 1, that is, they increase as the plant grows older. This is particularly striking in the chaff (table 2), in which the percentage of ash in the lignin at maturity was more than 50.

The percentages of lignin in the roots, as well as the percentages of nitrogen, methoxyl, and ash in the lignin, are given in table 4. The percentages of lignin and methoxyl in the lignin of the roots are shown graphically in figures 8 and 3 *B*, respectively. These constituents in the roots show the same general trend as those in the culms, sheaths, and leaves, that is, the percentages of lignin and methoxyl in the lignin increase, while the percentage of nitrogen in the lignin decreases as the plants grow older. The figures on the percentages of ash in the lignin from the roots are rather erratic because, as already explained, it was not possible to free the roots entirely from inorganic material.

PROTEIN EXTRACTED BY HOT AND COLD WATER

Table 5 gives the percentages of crude protein in the cold- and hot-water extracts (expressed also as the percentage of the total crude protein) in the culms, sheaths, and leaves, the chaff, the rachises and branches of the panicles, and the roots, respectively, at successive stages of growth.

Table 5 shows that the percentages of protein extracted from the culms, sheaths, and leaves and the roots by both cold and hot water followed the same general trend as those of the total crude protein, that is, after an initial increase they decreased as the plant grew older. This decrease, however, was not always regular. The data on the rachises and branches of the panicles show no initial increase in the percentage of crude protein extracted by cold water, but the percentage extracted by hot water follows the same trend as that extracted from the culms, sheaths, and leaves and the roots. With few exceptions, the protein extracted by the cold water, expressed as percentage of the plant material and as percentages of the total crude protein, was considerably greater than that removed by the hot water. The percentages of protein extracted from the chaff by the cold and hot water were rather irregular. In some instances, a greater percentage of protein was extracted by the cold water, while in others this was reversed.

TABLE 5.—*Crude protein in cold- and hot-water extracts of the various parts of oat plants at successive stages of growth*

[Calculated on basis of moisture-free and ash-free plant material]

CULMS, SHEATHS, AND LEAVES

Harvest No.	Age of plants	Total crude protein	Crude protein extracted by—			
			Cold water		Hot water	
			Percent	Percent of total	Percent	Percent of total
1.....	Days 7	Percent 38.12	2.12	5.56	2.37	6.22
2.....	14	41.50	2.19	5.28	2.99	7.20
3.....	21	42.69	5.29	12.39	2.48	5.81
4.....	28	45.00	10.56	23.47	1.94	4.31
5.....	35	41.19	8.62	20.93	2.12	5.15
6.....	42	31.00	3.86	12.45	1.60	5.16
7.....	49	19.31	2.86	14.81	1.23	6.37
8.....	56	14.88	2.10	14.11	.68	4.57
9.....	63	8.12	1.40	17.24	.60	7.39
10.....	70	5.75	.54	9.39	.54	9.39
11.....	77	5.31	.43	8.10	.47	8.85
12.....	84	4.00	.40	10.00	.31	7.75
13, lower third.....	91	2.19	.32	14.61	.16	7.30
13, middle third.....	91	2.75	.36	13.09	.18	6.54

TABLE 5.—Crude protein in cold- and hot-water extracts of the various parts of oat plants at successive stages of growth—Continued

CULMS, SHEATHS, AND LEAVES—Continued

Harvest No.	Age of plants	Total crude protein	Crude protein extracted by—			
			Cold water		Hot water	
	Days	Percent	Percent	Percent of total	Percent	Percent of total
13, upper third.....	91	3.00	.16	5.33	.21	7.00
14, lower third.....	98	2.31	.26	11.26	.21	9.09
14, middle third.....	98	1.88	.23	12.23	.23	12.23
14, upper third.....	98	2.19	.22	10.04	.22	10.04
15, lower third.....	105	1.75	.22	12.57	.22	12.57
15, middle third.....	105	2.12	.25	11.79	.20	9.43
15, upper third.....	105	2.44	.37	15.16	.21	8.61

CHAFF

9.....	63	12.56	1.29	10.27	0.71	5.65
10.....	70	13.38	1.05	7.85	1.05	7.85
11.....	77	13.44	.64	4.76	.77	5.73
12.....	84	9.94	.62	6.24	.74	7.44
13.....	91	7.62	.35	4.59	.62	8.14
14.....	98	5.38	.45	8.36	.50	9.29
15.....	105	5.88	.50	8.50	.50	8.50

RACHISES AND BRANCHES OF THE PANICLES

9.....	63	10.81	2.16	19.98	0.63	5.83
10.....	70	9.06	1.69	18.65	.64	7.06
11.....	77	9.19	.59	6.42	.59	6.42
12.....	84	7.56	.48	6.35	.44	5.82
13.....	91	4.88	.31	6.35	.31	6.35
14.....	98	4.25	.28	6.39	.24	5.65
15.....	105	4.00	.32	8.00	.27	6.75

ROOTS

1 ¹	7	13.69	0.96	7.01	0.82	5.99
2 ¹	14					
3.....	21	16.69	2.77	16.60	1.17	7.01
4.....	28	13.62	2.66	19.53	1.03	7.56
5.....	35	14.25	1.77	12.42	1.00	7.02
6.....	42	8.88	.99	11.15	.66	7.43
7.....	49	6.81	.89	13.07	.63	9.25
8.....	56	5.06	.58	11.46	.48	9.49
9.....	63	7.81	1.23	15.75	.50	6.40
10.....	70	3.50	.52	14.86	.36	10.28
11.....	77	3.06	.56	18.30	.22	7.19
12.....	84	2.31	.19	8.22	.19	8.22
13.....	91	2.19	.26	11.87	.13	5.94
14.....	98	2.00	.18	9.00	.12	6.00
15.....	105	1.88	.29	15.42	.17	9.04

¹ Samples from harvests 1 and 2 were combined and analyzed.

FURFURAL YIELDED BY SEVERAL COMPONENTS OF THE OAT PLANT

Table 6 shows the percentages of furfural yielded by several components of the culms, sheaths, and leaves, the chaff, the rachises and branches of the panicles, and the roots, respectively, of the oat plants at successive stages of growth. The figures in the third column of the table are taken from tables 1-4, respectively, and are inserted here for the sake of completeness and comparison. The figures in the fourth column were obtained by dividing by 4.60 the percentages of uronic acid anhydrides (ash-free basis) recorded in tables 1-4. The figures in the sixth column were obtained by multiplying the percentage of Cross and Bevan cellulose (ash-free basis) by the percentage of furfural in the Cross and Bevan cellulose (using the corresponding data

in tables 1-4), and dividing the result by 100. The figures in the eighth column represent the difference between the percentage of total furfural (third column) and the sum of the percentage of furfural from the uronic acid anhydrides and that from the pentose fraction of Cross and Bevan cellulose (fourth and sixth columns, respectively). The

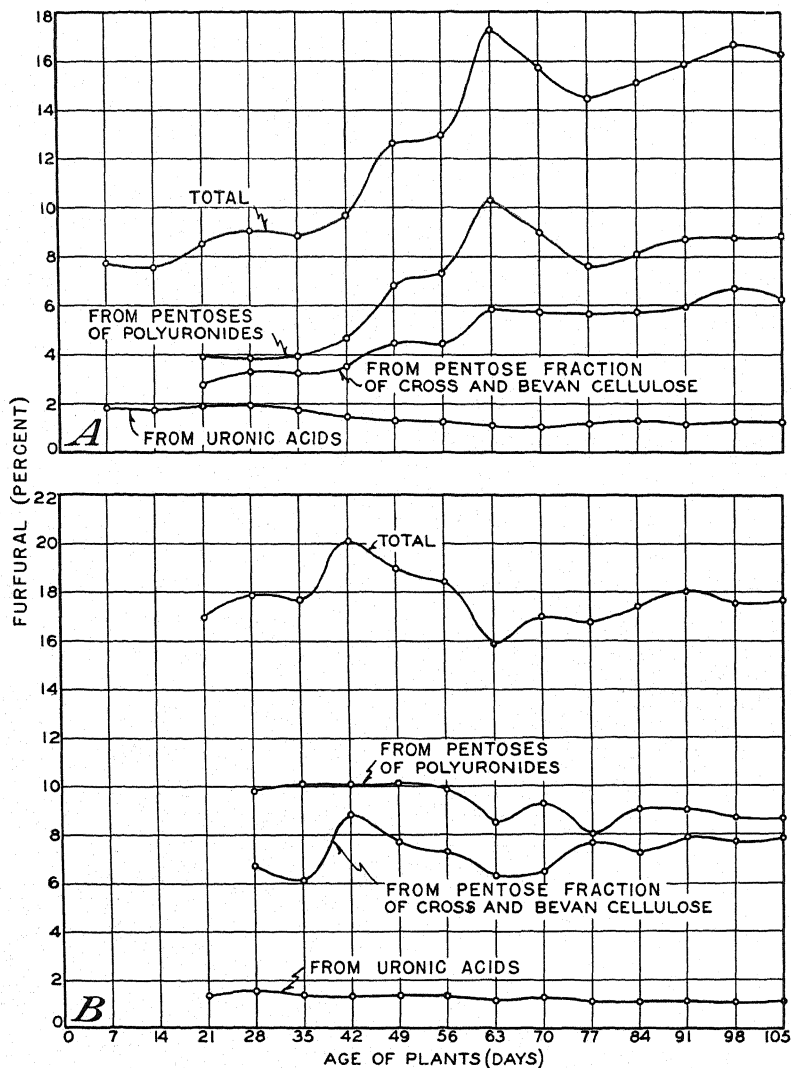


FIGURE 9.—Percentage of furfural yielded by several components of (A) the culms, sheaths, and leaves and (B) the roots of oat plants at successive stages of growth. (All values are on a moisture-free and ash-free basis.)

figures in the last column were obtained in each case by multiplying the percentage of furfural from the pentoses of the polyuronides (eighth column) by the factor 1.736. The data recorded in table 6 on the culms, sheaths, and leaves and the roots are shown graphically in figure 9.

TABLE 6.—Furfural yielded by several components of the various parts of the oat plant at successive stages of growth

[Calculated on basis of moisture-free and ash-free plant material]

CULMS, SHEATHS, AND LEAVES

Harvest No.	Age of plants	Total furfural	Furfural from—						
			Uronic acids		Pentose of Cross and Bevan cellulose		Pentoses of polyuronides		Pentoses of polyuronides calculated as xylose
	Days	Percent	Percent	Percent of total	Percent	Percent of total	Percent	Percent of total	Percent
1	7	7.74	1.81	23.39					
2	14	7.56	1.72	22.75					
3	21	8.54	1.86	21.78	2.78	32.55	3.90	45.67	6.77
4	28	9.03	1.90	21.04	3.28	36.32	3.85	42.64	6.68
5	35	8.86	1.72	19.41	3.24	36.57	3.90	44.02	6.77
6	42	9.69	1.45	14.96	3.56	36.74	4.68	48.30	8.12
7	49	12.62	1.30	10.30	4.46	35.34	6.86	54.36	11.91
8	56	13.00	1.21	9.31	4.44	34.15	7.35	56.54	12.76
9	63	17.25	1.09	6.32	5.85	33.91	10.31	59.77	17.90
10	70	15.78	1.02	6.46	5.78	36.63	8.98	56.91	15.59
11	77	14.48	1.19	8.22	5.66	39.09	7.63	52.69	13.25
12	84	15.15	1.28	8.45	5.79	38.22	8.08	53.33	14.03
13, lower third	91	15.70	1.24	7.90	6.46	41.15	8.00	50.95	13.89
13, middle third	91	15.50	1.16	7.48	5.87	37.87	8.47	54.65	14.70
13, upper third	91	16.44	1.16	7.06	5.55	33.76	9.73	59.18	16.89
14, lower third	98	16.46	1.24	7.53	6.89	41.86	8.33	50.61	14.46
14, middle third	98	16.52	1.23	7.45	6.59	39.89	8.70	52.66	15.10
14, upper third	98	17.13	1.24	7.24	6.62	38.65	9.27	54.11	16.09
15, lower third	105	15.87	1.31	8.25	6.58	41.47	7.98	50.28	13.85
15, middle third	105	15.98	1.20	7.51	6.14	38.42	8.64	54.07	15.00
15, upper third	105	17.11	1.16	6.78	6.09	35.59	9.86	57.63	17.12

CHAFF

9.....	63	21.12	1.28	6.06	6.02	28.50	13.82	65.44	23.99
10.....	70	17.70	1.17	6.61	5.14	29.04	11.39	64.35	19.77
11.....	77	17.26	1.00	5.79	5.94	34.42	10.32	59.79	17.92
12.....	84	17.20	1.03	5.99	5.16	30.00	11.01	64.01	19.11
13.....	91	17.70	1.14	6.44	5.65	31.92	10.91	61.64	18.94
14.....	98	19.60	1.17	5.97	5.44	27.76	12.99	66.27	22.55
15.....	105	19.76	1.13	5.72	5.49	27.78	13.14	66.50	22.81

RACHISES AND BRANCHES OF THE PANICLES

9.....	63	20.48	1.25	6.10	6.12	29.88	13.11	64.02	22.76
10.....	70	18.83	1.04	5.52	6.24	33.14	11.55	61.34	20.05
11.....	77	17.02	1.00	5.88	6.15	36.13	9.87	57.99	17.13
12.....	84	16.68	.96	5.76	6.09	36.51	9.63	57.73	16.72
13.....	91	19.10	1.06	5.55	6.21	32.51	11.83	61.94	20.54
14.....	98	19.44	1.02	5.25	7.55	38.84	10.87	55.91	18.87
15.....	105	19.78	1.03	5.21	7.54	38.12	11.21	56.67	19.46

ROOTS

1.....	7								
2.....	14								
3.....	21	16.99	1.39	8.18					
4.....	28	17.90	1.52	8.49	6.58	36.76	9.80	54.75	17.01
5.....	35	17.67	1.39	7.87	6.12	34.63	10.16	57.50	17.64
6.....	42	20.15	1.25	6.20	8.82	43.77	10.08	50.03	17.50
7.....	49	19.00	1.26	6.63	7.65	40.26	10.09	53.11	17.52
8.....	56	18.43	1.26	6.84	7.35	39.88	9.82	53.28	17.05
9.....	63	15.95	1.18	7.40	6.29	39.44	8.48	53.16	14.72
10.....	70	17.06	1.24	7.27	6.49	38.04	9.33	54.69	16.20
11.....	77	16.81	1.13	6.72	7.65	45.51	8.03	47.77	13.94
12.....	84	17.47	1.17	6.70	7.25	41.50	9.05	51.80	15.71
13.....	91	18.07	1.13	6.25	7.92	43.83	9.02	49.92	15.66
14.....	98	17.60	1.10	6.25	7.78	44.20	8.72	49.55	15.14
15.....	105	17.73	1.16	6.54	7.86	44.33	8.71	49.13	15.12

Table 6 and figure 9, *A*, show that after a slight variation during the early development of the plants the percentage of furfural furnished by the uronic acids of the culms, sheaths, and leaves remained fairly constant as the plants grew older and matured. When the plants were young about 23 percent of the total furfural was yielded by the uronic acids. In the later stages of the development of the plants, however, this had decreased considerably so that it ranged only from 6.78 to 8.25 percent. Similar results were obtained in previous studies with the barley plant (38). It will be observed from column 5, that in the samples from harvests 13, 14, and 15, the percentage of furfural from the lower, middle, and upper thirds followed in every case a regular descending order. The percentage of furfural yielded by the Cross and Bevan cellulose increased, though not regularly, as the plant grew older and matured. As in the case of the furfural from the uronic acids, the percentage of furfural decreased in the main, from the bottom of the stalk upward (harvest Nos. 13, 14, and 15). The percentage of furfural yielded by the pentoses of the polyuronides increased at first. It reached a maximum in the sample from the ninth harvest period, and subsequently declined somewhat, though not regularly, as the plant matured. The percentages of furfural from the pentoses of the polyuronides in the samples from the lower, middle, and upper thirds of harvests 13, 14, and 15 varied considerably, but here the percentage of furfural increased regularly from the lower third of the stalk upward. Throughout the development of the plant, the greatest percentage of the total furfural is furnished by the pentoses of the polyuronides. The figures in the last column show, of course, the same tendency as those recorded in the eighth column.

Table 6 shows that the percentages of furfural yielded by the uronic acids, the pentose fraction of Cross and Bevan cellulose, and the pentoses of the polyuronides of the chaff varied little with the increase in the age of the plant. Here, also, the greatest percentage of the total furfural was derived from the pentoses of the polyuronides; at maturity this amounted to more than 66 percent of the total furfural.

The percentage of furfural yielded by the uronic acids of the rachises and branches of the panicles decreased slightly, whereas the percentage of furfural from the Cross and Bevan cellulose increased, although not regularly, as the plants matured. The percentage of furfural derived from the pentoses of the polyuronides decreased somewhat as the plants matured. At maturity more than 56 percent of the total furfural was furnished by the pentoses of the polyuronides. In the plants from harvest No. 9, this amounted to more than 64 percent.

Table 6 and figure 9, *B*, show that the furfural yielded by the uronic acids from the roots decreased slightly as the plants grew older, while the percentage of furfural from the pentose fraction of Cross and Bevan cellulose was rather irregular. After an initial increase, it decreased, and finally as the plants matured it again increased somewhat. The percentage of furfural yielded by the pentoses of the polyuronides from the roots increased at first, and then decreased a little as the plants matured. In this case also, the greatest percentage of the total furfural was furnished by the pentoses of the polyuronides.

DISTRIBUTION OF FIRMLY BOUND METHOXYL GROUPS BETWEEN LIGNIN AND NONLIGNIN CONSTITUENTS

The distribution of the firmly bound methoxyl groups between the lignin and the nonlignin constituents in various parts of the oat plant at successive stages of growth is shown in table 7. The table shows that the lignin methoxyl in 100 gm. of plant material, as well as the percentage of lignin methoxyl calculated on the basis of the total firmly bound methoxyl, in the culms, sheaths, and leaves increased as the plant grew and matured. To be sure, this increase was not always regular, but the figures, in general, show an upward trend. In the fully mature plant, more than 80 percent of the total firmly bound methoxyl in the culms, sheaths, and leaves was found in the lignin. In the early stages of the development of the plant, the methoxyl not in the lignin, expressed as percentage of the total firmly bound methoxyl, was rather high, but the percentage of this decreased, in the main, as the plant grew older and matured. It has been shown by O'Dwyer (34), Schmidt and his coworkers (44), and by Hägglund and Sandelin (18) that in wood some of the firmly bound methoxyl groups are found also in the carbohydrate fraction. Anderson and Otis (1) identified methoxy glucuronic acid as one of the constituents of mesquite gum. Sands and Gary (42) and Sands and Nutter (43) showed that the hemicelluloses of mesquite wood contained the methoxyl group and that this group was associated with the fragment of hemicellulose that contained the uronic acid. Kurth and Ritter (23) found 3.2 percent of methoxyl in spruce "holocellulose," which was presumably freed entirely of lignin.

The lignin methoxyl in 100 gm. of plant material and the percentage of lignin methoxyl calculated on the basis of the total firmly bound methoxyl in the chaff and in the rachises and branches of the panicles did not increase very much as the plants grew and matured. At maturity, practically 90 and 85 percent, respectively, of the total firmly bound methoxyl was with the lignin.

The lignin methoxyl in 100 gm. of the roots increased almost regularly as the plants grew older. In comparing these data with those for the culms, sheaths, and leaves, it will be observed that in the roots the lignin methoxyl in 100 gm. of plant material was greater throughout the entire life of the plant. The lignin methoxyl, expressed as percentage of the total firmly bound methoxyl, ranged from 70.7 (harvest No. 3) to 87.0 (when the plants were fully mature).

WEIGHTS OF PLANTS AT DIFFERENT STAGES OF GROWTH

The weights of the culms, sheaths, and leaves, the chaff, the rachises and branches of the panicles, and the roots actually harvested and also the weights calculated on the basis of 100 plants are shown in table 8. The results present no special features. In the early stages of the development of the plants, the increase in weight in the culms, sheaths, and leaves was very rapid so that by the end of the eighth harvest period it amounted to approximately 500 times the weight at the end of the first week. The weight of the roots also increased, although to a smaller extent. In the chaff and in the rachises and branches of the panicles, the weight of dry matter increased at first, but later as maturity approached it decreased. This was presumably due to a translocation of some of the constituents from the chaff and panicles to the seed.

TABLE 7.—*Distribution of firmly bound methoxyl between lignin and nonlignin constituents of oat plants at successive stages of growth*
 [Results calculated on basis of moisture-free and ash-free plant material]

Harvest No.	Age of plant	Culms, sheaths, and leaves				Chaff				Rachises and branches of panicles				Roots			
		Firmly bound methoxyl	Lignin methoxyl	Methoxyl not in lignin	Firmly bound methoxyl	Lignin methoxyl	Methoxyl not in lignin	Firmly bound methoxyl	Lignin methoxyl	Methoxyl not in lignin	Firmly bound methoxyl	Lignin methoxyl	Methoxyl not in lignin	Firmly bound methoxyl	Lignin methoxyl	Methoxyl not in lignin	Percent of total
	Days	Grams ¹	Grams ¹	Percent of total	Grams ¹	Grams ¹	Percent of total	Grams ¹	Grams ¹	Percent of total	Grams ¹	Grams ¹	Percent of total	Grams ¹	Grams ¹	Percent of total	Percent of total
1.	7	0.17	0.09	52.9	18.3	0.85	17.5	1.20	1.07	82.9	2.52	2.12	84.1	2.52	2.12	84.1	22.7
2.	14	0.20	0.09	45.0	16.1	0.85	16.1	1.48	1.26	85.1	2.16	1.81	83.8	2.16	1.81	83.8	29.3
3.	21	0.28	0.11	39.3	16.1	0.85	16.1	1.47	1.23	83.7	2.28	1.88	83.3	2.28	1.88	83.3	20.6
4.	28	0.29	0.13	44.8	15.2	0.81	15.2	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	26.1
5.	35	0.25	0.13	52.0	14.8	0.81	14.8	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	20.9
6.	42	0.29	0.15	51.7	14.8	0.81	14.8	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	15.2
7.	49	0.30	0.15	50.0	14.8	0.81	14.8	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	18.4
8.	56	0.30	0.15	50.0	14.8	0.81	14.8	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	17.0
9.	63	0.30	0.15	50.0	14.8	0.81	14.8	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	16.2
10.	70	0.30	0.15	50.0	14.8	0.81	14.8	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	10.8
11.	77	0.30	0.15	50.0	14.8	0.81	14.8	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	25.8
12.	81	0.30	0.15	50.0	14.8	0.81	14.8	1.56	1.28	82.1	2.29	1.70	74.2	2.29	1.70	74.2	15.9
13.	91	2.11	1.22	85.6	12.3	0.99	85.3	1.59	1.39	87.4	2.52	2.12	84.1	2.52	2.12	84.1	18.5
13, upper third.		2.01	1.22	85.6	12.3	0.99	85.3	1.59	1.39	87.4	2.52	2.12	84.1	2.52	2.12	84.1	18.5
13, lower third.		1.79	1.37	57.7	12.3	0.99	85.3	1.59	1.39	87.4	2.52	2.12	84.1	2.52	2.12	84.1	18.5
14.	98	2.54	1.85	80.8	19.2	1.05	89.0	1.71	1.51	88.3	2.65	2.16	81.5	2.65	2.16	81.5	13.0
14, upper third.		2.20	1.74	78.6	19.2	1.05	89.0	1.71	1.51	88.3	2.65	2.16	81.5	2.65	2.16	81.5	13.0
14, middle third.		2.30	1.95	84.6	19.2	1.05	89.0	1.71	1.51	88.3	2.65	2.16	81.5	2.65	2.16	81.5	13.0
15.	105	2.39	1.95	83.3	18.7	1.04	88.9	1.72	1.46	84.9	2.62	2.28	87.0	2.62	2.28	87.0	13.0
15, upper third.		2.33	1.91	81.9	18.7	1.04	88.9	1.72	1.46	84.9	2.62	2.28	87.0	2.62	2.28	87.0	13.0
15, middle third.		2.13	1.74	81.7	18.3	1.04	88.9	1.72	1.46	84.9	2.62	2.28	87.0	2.62	2.28	87.0	13.0

¹ In 100 gm. of plant material.

² Samples from harvests 1 and 2 were combined and analyzed.

TABLE 8.—*Weight of plants at different stages of growth*

[Calculated on moisture-free basis]

Harvest No.	Age of plants	Height of plants	Plants harvested	Weight of plants harvested				Weight of 100 plants						
				Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total	
	Days	Centi- meters	Number	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Total
1. 14, lower third. 2. 14, middle third. 3. 14, upper third. 4. 15, lower third. 5. 15, middle third. 6. 15, upper third. 7. 16, lower third. 8. 16, middle third. 9. 16, upper third. 10. 17, lower third. 11. 17, middle third. 12. 17, upper third. 13. 18, lower third. 14. 18, middle third. 15. 18, upper third. Total 1. Total 2.	1	7-10	1,020	22.34	2.32	24.66	2.32	24.66	2.19	0.23	2.42			
	2	11	8-11	524	31.21	3.56	34.77	3.56	34.77	5.95	0.68	6.63		
	3	14	923	140.52	18.79	168.31	18.79	168.31	16.20	2.03	18.23			
	4	21	923	307.27	38.77	346.04	38.77	346.04	49.56	6.25	55.81			
	5	28	620	370.43	39.74	410.17	39.74	410.17	128.62	13.80	142.42			
	6	35	288	722.18	61.84	784.02	61.84	784.02	299.66	25.66	325.32			
	7	42	241	958.93	72.93	1,031.86	72.93	1,031.86	384.71	44.47	629.18			
	8	49	164	1,043.18	94.71	1,137.89	94.71	1,137.89	1,003.06	91.07	1,194.13			
	9	56	104	959.50	99.11	1,058.61	111.36	1,170.00	1,370.71	141.58	1,511.59			
	10	63	70	1,051.98	132.18	1,184.16	89.88	1,274.04	1,783.02	224.03	1,998.06			
	11	70	59	1,023.24	77.14	1,100.38	85.84	1,186.22	1,677.44	126.46	1,803.90			
	12	77	61	1,903.92	85.82	1,989.74	68.97	2,058.71	1,807.84	171.64	2,237.38			
	13	84	50	208.41	51.53	259.94	47.78	306.69	413.11	117.11	530.22			
	14	91	44	181.77	---	---	---	---	455.25	---	---			
	14, lower third. 14, middle third. 14, upper third. Total 1. Total 2.				590.49	---	---	---	590.49	1,342.02	---	---	---	---
				211.28	---	---	---	211.28	571.02	136.76	94.97	101.16	1,1763.07	
				202.18	50.60	252.78	37.43	290.21	546.43	---	---	---	---	
				115.71	---	---	---	115.71	312.73	---	---	---	---	
				529.17	---	---	---	529.17	1,430.18	---	---	---	---	
				295.77	---	---	---	295.77	739.42	134.97	82.00	120.62	1,2256.74	
				294.86	53.99	348.85	48.25	397.11	662.15	---	---	---	---	
				207.03	---	---	---	207.03	517.58	---	---	---	---	
				797.66	---	---	---	797.66	1,919.15	---	---	---	---	
				---	---	---	---	---	---	---	---	---	---	
				---	---	---	---	---	---	---	---	---	---	
				---	---	---	---	---	---	---	---	---	---	
				---	---	---	---	---	---	---	---	---	---	
				---	---	---	---	---	---	---	---	---	---	

¹ Does not include weight of the grain.² Total weights of culms, sheaths, and leaves.

WEIGHTS OF DIFFERENT CONSTITUENTS AT SUCCESSIVE STAGES OF
DEVELOPMENT OF THE PLANT

The weights of the more important constituents of the several parts of the oat plant at successive stages of growth are shown in table 9. In all cases, the results were calculated on the basis of 100 plants.

The data on the ash call for no special comment. There was a constant increase in the actual quantity of ash in each part during the greatest portion of the life of the plants. In the later stages of development, there was a decrease in the quantity of ash, due presumably to a translocation of the inorganic matter, in part at least, to the seed. At maturity, the ash content of certain parts of the plant again increased somewhat.

The quantity of crude protein in all parts of the oat plant except the rachises and branches of the panicle increased rapidly during the first part of the life of the plant, and then decreased steadily, although not always regularly, as the plant developed and matured. In general, the quantity of crude protein in the rachises and branches of the panicle decreased during the development of the plant.

In the total plant the content of total methoxyl as well as the methoxyl in the extracted plant material increased, although not regularly, as the plant developed and matured.

TABLE 9.—Weight of various constituents in 100 oat plants at different stages of growth

Harvest No.	Age of plants Days	Ash in—					Crude protein in—					Total methoxyl in—				
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants
		Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
1	7	0.35					0.70				5.97	0.02			0.04	0.17
2	14	1.00				3.13	2.06				19.43	.15			.09	1.46
3	21	2.91				8.99	5.67				46.89	.40			.21	2.99
4	28	8.02				20.62	18.71				80.48	2.29			.47	7.52
5	35	18.92				49.71	45.17				151.13	6.59			.97	17.75
6	42	46.63				83.96	78.48				182.92	13.60			2.10	39.86
7	49	78.14				122.66	96.83				182.92	20.00			4.37	56.96
8	56	103.82				184.22	96.77				182.92	23.60			4.87	55.27
9	63	141.04				221.40	94.68				182.92	23.60			4.87	55.27
10	70	181.16				224.62	79.68				182.92	23.60			4.87	55.27
11	77	211.16				260.67	64.36				182.92	23.60			4.87	55.27
12	84	188.77				176.68	31.77				182.92	23.60			4.87	55.27
13	91	150.76				189.71	27.06				182.92	23.60			4.87	55.27
14	98	203.35				253.00	35.32				182.92	23.60			4.87	55.27
15	105										182.92	23.60			4.87	55.27

Harvest No.	Age of plants and days	Hot-water extracts in—					1-percent hydrochloric acid extracts in—					Total extractives in—				
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants
		Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
1	7	0.03	—	—	—	—	0.24	—	—	—	—	1.12	—	—	—	—
2	14	.10	—	—	—	—	0.39	—	—	—	—	3.02	—	—	—	—
3	21	.26	—	—	0.08	0.32	1.64	—	—	0.54	2.18	6.97	—	—	0.91	7.88
4	28	.59	—	—	.18	.77	6.21	—	—	1.81	8.02	20.75	—	—	2.54	23.29
5	35	2.03	—	—	.43	2.46	15.14	—	—	3.72	18.86	50.43	—	—	5.86	56.29
6	42	4.07	—	—	.60	5.27	39.26	—	—	7.70	46.96	139.28	—	—	10.47	149.75
7	49	8.71	—	—	.86	9.37	99.24	—	—	13.45	112.79	271.36	—	—	16.96	288.32
8	56	18.03	—	—	2.18	20.24	210.24	—	—	21.86	232.10	491.70	—	—	32.06	523.76
9	63	15.63	1.91	2.15	3.53	23.22	318.42	40.79	32.08	39.68	439.97	575.01	71.65	45.08	63.05	764.79
10	70	20.15	4.37	1.95	3.17	29.64	398.50	68.64	29.44	43.51	510.09	792.37	111.41	45.99	60.04	1,009.81
11	77	17.28	2.91	2.98	2.90	26.07	376.42	36.42	34.55	37.63	495.10	780.05	60.09	52.98	53.85	926.97
12	84	35.80	6.13	3.98	2.83	48.74	415.98	50.51	37.37	39.84	538.70	781.17	83.04	58.76	52.75	975.72
13	91	19.64	7.50	1.55	2.43	31.12	333.95	33.60	25.86	33.94	427.36	580.45	57.24	40.57	40.47	698.73
14	98	15.86	5.03	2.02	2.12	25.03	375.98	41.26	29.87	31.20	478.31	543.61	61.84	43.43	40.20	680.11
15	105	23.07	5.78	1.42	1.24	31.51	498.98	39.24	24.29	36.19	598.70	704.91	62.80	38.04	46.47	882.22

TABLE 9.—Weight of various constituents in 100 oat plants at different stages of growth—Continued

Harvest No.	Age of plants	Uronic acids (as anhydrides) in—					Total furfural yielded by—					Pentosans in—				
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants
	Days	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
1	7	0.15				0.14						0.19				
2	14	39				.37						1.52				2.00
3	21	1.13			0.12	1.25	1.13			0.31	1.44	5.06			0.48	6.54
4	28	3.64			.37	4.01	3.75			.94	4.69	13.38			1.48	16.75
5	35	8.69			.77	9.46	9.71			2.14	11.85	35.08			3.37	42.96
6	42	16.90			1.30	18.20	24.54			4.55	29.09	97.06			7.30	108.79
7	49	29.94			2.24	32.18	63.27			7.35	70.62	181.25			11.73	202.46
8	56	49.95			4.19	54.14	116.86			13.31	130.17	327.87			21.21	337.05
9	63	60.04	7.76	5.23	7.38	80.41	204.78	27.96	18.68	21.62	273.04	414.73	44.94	29.99	34.25	437.05
10	70	77.03	11.07	4.44	7.71	100.25	250.25	36.34	17.46	23.03	336.08	414.73	58.05	28.20	36.52	537.90
11	77	82.19	5.16	4.83	6.19	98.37	216.73	19.04	17.71	20.15	273.63	340.18	30.64	28.48	32.14	431.44
12	84	94.37	6.93	4.90	6.68	112.88	241.89	25.08	18.58	21.52	307.07	378.74	40.30	29.93	34.32	483.29
13	91	65.73	5.11	3.67	5.13	79.64	191.23	17.33	14.43	17.86	240.85	302.56	27.73	23.32	25.58	332.23
14	98	72.59	6.04	4.08	4.84	87.55	212.83	21.95	16.55	16.77	268.40	336.03	35.28	27.29	26.88	426.38
15	105	97.10	5.72	3.54	5.86	112.22	277.81	21.78	14.66	19.42	333.67	438.98	35.13	23.76	31.02	528.89

Harvest No.	Age of plants and of plants	Cross and Bevan cellulose in—					Cross and Bevan xylan ¹ in—					Cellulose in—				
		Culm, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Culm, sheaths, and leaves	Chaff	Rachises and branches of panicles	Total plants	Culm, sheaths, and leaves	Chaff	Rachises and branches of panicles	Roots	Total plants	Grams
1.	7	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
2.	14	3.40	—	—	—	13.46	0.58	—	—	—	2.84	—	—	1.98	10.80	—
3.	21	10.95	—	—	2.51	35.37	2.13	—	—	—	8.82	—	—	4.26	28.70	—
4.	28	29.97	—	—	5.40	90.12	5.52	—	—	—	24.44	—	—	8.83	72.96	—
5.	35	78.21	—	—	11.91	187.26	14.02	—	—	—	64.13	—	—	14.27	147.58	—
6.	42	168.40	—	—	18.86	362.96	34.79	—	—	—	133.31	—	—	28.78	292.58	—
7.	49	325.99	—	—	36.97	672.42	61.89	—	—	—	293.80	—	—	50.27	531.37	—
8.	56	526.35	47.71	34.88	63.48	107.33	107.33	12.33	8.64	13.22	419.44	35.40	25.26	51.95	687.21	—
9.	63	704.29	66.54	35.97	65.51	147.46	147.46	16.38	8.98	13.62	558.08	50.18	27.00	43.45	598.31	—
10.	70	629.04	37.43	37.31	59.66	763.44	131.34	10.18	9.95	14.23	498.20	27.32	27.34	49.52	673.72	—
11.	77	705.06	45.83	40.37	63.31	854.57	143.18	11.65	10.55	13.86	592.24	34.16	29.80	39.85	540.84	—
12.	84	557.72	33.14	30.04	52.01	672.91	111.32	8.60	7.28	12.15	453.64	24.59	22.76	36.62	603.10	—
13.	91	638.95	40.62	39.79	48.15	707.51	133.40	9.46	10.14	11.51	505.67	31.18	29.03	42.10	780.28	—
14.	98	849.46	39.55	34.28	55.48	978.77	167.12	9.41	8.66	13.35	682.41	30.10	25.67	—	—	—
15.	105	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

¹ Xylan fraction associated with Cross and Bevan cellulose.

TABLE 9.—Weight of various constituents in 100 oat plants at different stages of growth—Continued

Harvest No.	Age of plants Days	Reducing sugars ² (as dextrose) in—			Nonreducing sugars ¹ (as sucrose) in—			Lignin in—			Total plants
		Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	Culms, sheaths, and leaves	Chaff	Rachises and branches of panicles	
		Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams	Grams
1	7	—	—	—	—	—	—	0.03	—	—	0.35
2	14	—	—	—	—	—	—	.11	—	—	1.33
3	21	0.15	—	—	0.29	—	—	.25	—	—	3.19
4	28	.47	—	—	.24	—	—	1.01	—	—	7.64
5	35	2.53	—	—	2.21	—	—	2.46	—	—	21.96
6	42	8.30	—	—	11.54	—	—	6.14	—	—	53.71
7	49	16.20	—	—	17.89	—	—	18.94	—	—	120.11
8	56	54.87	—	—	52.56	—	—	47.94	—	—	173.03
9	63	—	2.10	1.03	—	2.12	2.11	91.29	9.32	7.30	163.46
10	70	76.13	3.81	1.18	79.52	12.72	3.14	321.77	15.86	7.84	187.44
11	77	82.03	3.06	1.97	70.12	2.44	3.11	333.69	7.96	8.96	145.72
12	84	52.61	3.78	2.85	33.81	1.29	2.15	192.04	11.50	6.28	161.27
13	91	20.90	1.12	.69	30.85	1.66	1.53	131.00	7.90	8.04	13.74
14	98	4.83	.86	—	4.26	.36	—	131.72	9.71	—	—
15	105	6.15	.73	—	5.47	.31	—	187.31	9.76	—	—

² Not determined in roots.

The quantities of the various extractives in 100 plants recorded in table 9 may conveniently be considered together. In the earlier stages of the growth of the plants the weights of the extractives increased rapidly, but in the later stages they decreased. The only exception to this is the 1-percent hydrochloric acid extractives, for which the figures show, in the main, an upward trend.

In general, the uronic acids in the culms, sheaths, and leaves and the total in the plants increased as the plants grew older and matured, except in the materials from harvests 13 and 14. It will be noted throughout this table that the results from harvests 13 and 14 are, in every case, not in harmony with the rest of the data. However, the figures on the uronic acids in the chaff and the roots show at first an upward and later a downward trend. In the rachises and branches of the panicles there was a general decrease.

The content of furfural-yielding constituents in 100 plants, as well as the total pentosans, increased (with one or two exceptions) as the plants grew and developed. The greatest increase, of course, occurred in the culms, sheaths, and leaves.

The Cross and Bevan cellulose, xylan in Cross and Bevan cellulose, and cellulose increased irregularly as the plants grew and matured. The apparent decrease noted in the plants from harvests 13 and 14 is believed to be due to experimental error.

The data on the reducing and nonreducing sugars are incomplete because there was not enough material for analysis. The results presented on the content of these sugars in the culms, sheaths, and leaves, chaff, and rachises and branches of the panicles indicate that there was a rapid increase in the quantities of these sugars in the early stages of the development of the plants. As the plant grew older and matured, there was a rapid decline in the content of reducing and nonreducing sugars. No doubt to a considerable extent this was due to a translocation of the sugars from the culms and leaves to the seed, where they were stored in the form of starch.

The data on the lignin show that this constituent increased throughout the life of the plants. The data on the weight of lignin at different stages of the development of the plants when taken in conjunction with the data on cellulose are interesting. It will be observed that the actual quantities of both these components increased as the plants grew older, and therefore there is no direct evidence that the lignin increased at the expense of the cellulose.

DISCUSSION

Cross, Bevan, and Beadle (8, *p.* 179) consider that lignification is a process whereby there is a continuous modification of the cellulose, which is ultimately converted into lignin. In support of this hypothesis they state that "lignocelluloses in the first year of growth contain 70-80 p.ct. cellulose, the woods, on the other hand, 50-60 p.ct." As has already been pointed out elsewhere in this paper, results on percentage composition may be misleading in that they indicate the relative amount of each component but not the absolute quantity. A decrease in the percentage of cellulose does not necessarily mean that there was an actual disappearance of this substance, but rather that some of the other components increased at a much more rapid rate. The absolute quantity of cellulose present may have remained the

same or even may have increased somewhat. The results on the percentages and on the absolute quantities of lignin and cellulose obtained in this investigation show definitely that both these constituents increased as the plant developed and grew to maturity. There was no indication that the plant synthesized lignin at the expense of the cellulose. Moreover, considering the structure of cellulose and its function in the vegetable kingdom, it does not seem reasonable to suppose that it is used by the plant as an intermediate or as a starting material for the synthesis of other substances. There is every indication that cellulose is an end product, produced by the plant presumably from glucose, and is not utilized by it as the starting material for phytochemical syntheses. Moreover, the presence of a plant enzyme capable of bringing about a degradation of cellulose *in vivo* has never been demonstrated.

The theory that pectin is the parent substance utilized by the plant in the synthesis of lignin has a certain element of plausibility. Although no direct determination of pectin was made, the data on the content of uronic acids may give, indirectly, an indication of the quantity of this complex present at various stages of the development of the plant. It is realized, of course, that uronic acids are also present in the polyuronide hemicelluloses. However, while it is generally agreed that the percentage of uronic acid (as anhydride) in the pectins is about 70, it is only about one-third this percentage in the polyuronide hemicelluloses. Any appreciable loss of pectin caused by its conversion into lignin would necessarily reduce the uronic-acid content of the plant material, unless it be assumed that the synthesis of the polyuronide hemicelluloses is at once stimulated at such an accelerated rate as to compensate for the loss of pectin. While this is possible, the data obtained in this investigation definitely do not support this hypothesis. Attention is called again to the results of Buston (4) on the lignification of a rose shoot. Although there was a rapid increase in the lignin content, the absolute quantity of pectin did not decrease but rather increased somewhat. All in all, it may be stated that the claim that the plant synthesizes lignin from pectin is not supported by experimental results and seems, in fact, quite improbable.

The results obtained in this investigation do not support the contention of Rassow and Zschenderlein (40) that lignin is synthesized by the plant from pentosans. It will be observed (table 9) that the absolute quantities of both pentosans and lignin increased as the plants grew older and matured. There was no evidence that one was synthesized at the expense of the other.

If we agree that either sucrose or glucose is the first product of phytochemical synthesis, it is believed that the plant builds up lignin directly from either one of these sugars. Whether fructose alone is used in this synthesis, as claimed by Wislicenus (49), or whether both sugars are utilized for this purpose is, of course, not known. It may not be out of place to emphasize, however, that lignification is not, as is sometimes assumed, a senescent change. Lignin is produced even in the very earliest stages of the development of the plant. This is clear from the results obtained in this and in previous investigations (36, 38). Among the first steps in the synthesis of lignin is the production of a substance or substances having firmly bound (in etherlike combination) methoxyl groups. As was

pointed out by Browne and Phillips (3), these may be formed in the splitting up of carbohydrates, by a process of hydrolysis, oxidation, reduction, and dehydration and not necessarily directly from formaldehyde by a process of methylation. The presence (table 7) of a large quantity of substances, presumably carbohydrates, with firmly bound methoxyl groups, which gradually decrease with the increase in the content of lignin, lends support to this hypothesis. Whether these methoxyl-containing substances are then converted into methoxyl derivatives of the pentoses by a process of oxidation and decarboxylation (via the uronic acid stage), and these in turn are converted further into lignin, is at present not known. It appears quite certain, however, that coniferyl alcohol (or aldehyde) is not one of the intermediate products in the phytochemical synthesis of lignin. The results of this and previous investigations (36, 38) definitely do not support Klason's (20) hypothesis that the plant synthesizes lignin from coniferyl alcohol (or aldehyde) by a process of condensation or polymerization. If this were true one would expect the percentage of methoxyl in the lignin to remain substantially constant. The results of this investigation, however, show very conclusively that the percentage of methoxyl in the lignin is a variable quantity and that it increases markedly as the plant develops and matures (see tables 1, 4, and 7). In view of the results of the present investigation, Klason's hypothesis as to the mechanism involved in the phytochemical synthesis of lignin must be definitely rejected. It appears more probable that several intermediate substances are utilized by the plant in the synthesis of lignin. These substances do not contain the same percentage of methoxyl, and some may even be entirely free of this substituent. In the process of synthesis of lignin by the plant, these building units are combined in different proportions during the development of the plant. This would explain the fact that lignin isolated at successive stages of the development of the plant differs in its methoxyl content. Further research is required to establish the nature of the intermediate substances utilized by the plant in the synthesis of lignin.

SUMMARY AND CONCLUSIONS

The composition of the culms, sheaths, and leaves, chaff, rachises and branches of the panicle, and roots of the oat plant at successive stages of growth was studied with the object of determining the nature and percentage of the more important constituents, and thereby learning more about the formation of lignin by the growing plant and the possible mechanism involved in the process of lignification.

After an initial increase, the percentage of ash in the culms, sheaths, and leaves decreased, though irregularly, as the plant matured. The percentage of ash in the rachises and branches of the panicle showed a similar trend, while that of the chaff increased regularly. The percentage of ash in the roots was irregular. In the main, the absolute quantity of ash in the plant increased as the plant grew and matured.

The percentage of nitrogen (and crude protein) in the several parts of the oat plant studied, after an initial increase, decreased with maturity, with the exception of the chaff and the rachises and branches of the panicle, which showed a regular decrease. The absolute

quantity of nitrogen (and crude protein) increased rapidly during the early stages of the growth of the plant, and then decreased steadily, although not always regularly, as the plant developed further and matured.

The percentages, as well as the absolute quantities, of methoxyl in the original and in the extracted plant materials increased, in general, as the plant developed and matured.

In the earlier stages of growth of the plant, the weight of the various extractives increased rapidly, but in the later stage of its development it decreased somewhat. The only exception to this was the total 1-percent hydrochloric acid extractives, the figures for which show, in general, an upward trend.

After an initial increase, the percentage of uronic acids (as anhydrides) in the culms, sheaths and leaves, and the roots decreased slightly as the plant matured. In the chaff and in the rachises and branches of the panicle there was also a decrease, although an irregular one, as the plant grew older. The weight of the total uronic acids (as anhydrides) in the plant increased as the plant developed and grew to maturity.

The percentages of the total furfural and pentosans in the culms, sheaths, and leaves increased regularly and reached a maximum when the plant was 63 days old. Subsequently there was a decrease, but at or near maturity there was a slight increase. After a slight variation in the early development of the several parts of the plant studied, the percentage of furfural furnished by the uronic acids remained fairly constant. In the culms, sheaths, and leaves, the percentage of furfural yielded by the uronic acids, expressed as percentage of the total furfural, ranged from 23.4 in the early stages of growth to 6.8 at maturity. In the chaff and in the rachises and branches of the panicle, the percentage of furfural yielded by the uronic acids, expressed as percentage of the total furfural, ranged from about 6 in the early stages of growth to a little over 5 at maturity, while in the roots it ranged from about 8.5 to 6.25. The percentage of furfural yielded by the Cross and Bevan cellulose of the culms, sheaths, and leaves increased, though not regularly, as the plant grew older and matured. The percentage of furfural yielded by the pentoses of the polyuronides of the culms, sheaths, and leaves increased at first and then decreased irregularly. As the plant grew older, in each part except the roots more than 50 percent of the total furfural was furnished by the pentoses of the polyuronides. The weights of the total furfural-yielding substances and pentosans generally increased as the plant grew older and matured.

The percentages of Cross and Bevan cellulose, as well as the cellulose of the culms, sheaths, and leaves, and rachises and branches of the panicle, increased, but not regularly, as the plant grew older. In the roots the percentages of these constituents did not materially change. The percentage of xylan in the Cross and Bevan cellulose from the culms, sheaths, and leaves, rachises and branches of the panicle, and roots increased somewhat as the plant grew older, but the increase was neither consistent nor even. The weights of Cross and Bevan cellulose, xylan in the Cross and Bevan cellulose, and cellulose increased irregularly as the plant grew older.

The percentages of reducing and nonreducing sugars (calculated as glucose and sucrose, respectively) in the culms, sheaths, and leaves, chaff, and rachises and branches of the panicle increased in the early stages of growth and then decreased markedly as the plant grew older.

The percentage of lignin, as well as methoxyl in the lignin, in the culms, sheaths, and leaves, chaff, rachises and branches of the panicle, and roots generally increased as the plant developed and matured. The weight of total lignin also increased with the development and maturity of the plant.

In the fully mature plant, more than 80 percent of the total firmly bound methoxyl in the culms, sheaths, and leaves was found in the lignin. In the early stages of the development of the plant, the methoxyl not in the lignin, expressed as percentage of the total firmly bound methoxyl, was rather high, but the percentage of this decreased, in the main, as the plant grew older and matured. The lignin methoxyl, expressed as percentage of the total firmly bound methoxyl, in the roots ranged from 70.7 to 87.0.

The results obtained in this investigation indicate that the plant does not synthesize lignin from cellulose, pectin, or pentosans. The data are also not in harmony with Klason's hypothesis that coniferyl alcohol (or aldehyde) is the intermediate substance utilized by the plant in the synthesis of lignin. It is suggested that lignin is synthesized by the plant directly from either glucose or sucrose. Among the first steps in the synthesis of lignin is the production of a substance or substances having firmly bound (in etherlike combination) methoxyl groups, which may be formed in the course of splitting up carbohydrates by a process of hydrolysis, oxidation, reduction, and dehydration. The observed presence of large quantities of substances, presumably carbohydrates, containing firmly bound methoxyl groups and the gradual decrease of these substances with the increase in the content of lignin lends support to this hypothesis.

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A MOSAIC DISEASE OF CABBAGE¹

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INTRODUCTION

For several years a mosaic disease has been observed on cabbage (*Brassica oleracea capitata* L.) in southeastern Wisconsin, but not until 1934 did it become widely prevalent. Preliminary investigations of the disease started in that year have already been reported by Blank (4).³ The present writers took up the study in 1936 when the great destructiveness of the disease emphasized the need for more detailed information concerning the properties, vectors, and host range of the virus.

The masking of symptoms which may occur on cabbage has undoubtedly delayed recognition of the real severity of this disease. Its potential destructiveness was clearly emphasized in 1936 when widespread occurrence followed a heavy infestation of cabbage aphids which continued for the major part of the growing season. The yield from several thousand acres was reduced by approximately one-half, and the internal necrosis of heads which became evident at and following harvest resulted in still further losses.

The object of the present study was to record the symptoms of this disease on cabbage and other hosts, to determine the host range of the virus, to investigate the means of its transmission and overwintering, and to describe the virus as well as possible by a study of its properties. Preliminary reports have been published elsewhere (21, 44).

PREVIOUS WORK ON VIRUS DISEASES OF CRUCIFERS

The first reports of a transmissible virus disease on cruciferous plants were made in 1921, when, as a result of independent investigations in two laboratories, the mosaic disease of turnip (*Brassica rapa* L.) was described. Gardner and Kendrick (11) at the Indiana Experiment Station transferred, by mechanical inoculation, the infectious entity from naturally occurring mosaic-diseased turnips to plants of the same species. They did not secure infection on radish (*Raphanus sativus* L.). Schultz (28), working concurrently at the Bureau of Plant Industry laboratories in Washington, D. C., had brought to his attention by W. A. Orton mosaic-diseased plants of turnip, Chinese cabbage (*Brassica pekinensis* (Lour.) Gagn.), and pot-herb mustard (*B. japonica* (Thunb.) Sieb.). He found that the infectious entity could be transmitted interchangeably between the three species

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³ Italic numbers in parentheses refer to Literature Cited, p. 390.

either by mechanical inoculation or by means of the aphid vector, *Myzus persicae* (Sulz.). A mosaic disease of Chinese cabbage has since been reported in many parts of the world (1, 6, 8, 9, 15, 20, 23, 25, 35, 45), and recently the disease on this plant has been described fully from California by Tompkins and Thomas (42). Equally widespread have been reports of turnip and rutabaga mosaic (2, 5, 6, 10, 14, 13, 16, 26, 45), the former having been described most completely in a recent paper by Tompkins (40). Other reports of mosaic on crucifers before 1930 were on *Raphanus sativus caudatus* (L.) Bailey from India (19), and on an unnamed species of *Raphanus* and on charlock (*Brassica arvensis* (L.) Ktze.) from Denmark (13). Between 1926 and 1929 a number of cruciferous hosts to the sugar-beet curly top virus were reported from the Pacific coast (22, 29, 30).

As early as 1910, Stewart (34, p. 167) observed in New York fields a necrotic flecking of the outer and inner head leaves of cabbage. He did not suggest a virus as the inducing agency, but it may well have been that his observations were concerned with the effects of cabbage mosaic. A chemical study of a mosaic disease of cabbage was reported by Jodidi et al. (17) in 1920, although these workers were unable to demonstrate that they were working with an infectious virus disease. In 1924 Spierenburg (33) in Germany noted internal flecking of cabbage which resembled the symptoms of cabbage mosaic. Following the preliminary report of cauliflower mosaic in New York by Thatcher (36), Clayton (6) reported extensive cross-inoculation studies with the virus from mosaic-infected rutabaga on Long Island. He failed to secure infection on cabbage although he transmitted the virus to its sister subspecies, cauliflower and Brussels sprouts (*Brassica oleracea botrytis* L. and *gemmifera* DC.) and to several other crucifers. The first authentic transfer of a mosaic virus to cabbage was reported in 1934 by Tompkins (37) in the transfer of cauliflower mosaic to that host. In 1935 Hoggan and Johnson (16), who obtained inoculum from a naturally infected turnip collected in southeastern Wisconsin by the senior author of this paper and from horse-radish collected in Illinois, secured positive infection of cabbage. It was obvious by this time that probably several mosaic viruses of crucifers occurred, and the last-mentioned authors were the first to make studies of the properties of this group (16). In this same year the first natural occurrence of mosaic on cabbage was described from southeastern Wisconsin by Blank (4). This was followed by reports from Oregon⁴ and Washington.⁵ A ringspot virus disease of cabbage was described from England by Smith (32) in 1935, but this disease appears to be distinct from mosaic and more likely is similar to the disease reported later from California by Tompkins et al. (41).

The full account of the cauliflower mosaic of the Pacific coast (39) shows it to be distinct from the disease described in this paper. Among the other cruciferous hosts upon which the natural occurrence of mosaic has been described recently are wild radish (*Raphanus raphanistrum* L.) (43), rape (*Brassica napus* L.) (5, 18), colza (*B. napus* L. *oleifera* DC.) (18), horseradish⁶ (7), pe-tsai and Chinese mustard (2), honesty (*Lunaria annua* L.) (24), wallflower (*Cherianthus cheiri* L.)

⁴ McWHORTER, F. D. MOTTILING OR BREAKING IN DAME'S ROCKET IN OREGON. U. S. Bur. Plant Indus., Plant Dis. Repr. 20: 199. 1936.

⁵ JONES, LEON K. OBSERVATIONS ON PLANT DISEASES IN WASHINGTON IN 1936. U. S. Bur. Plant Indus., Plant Dis. Repr. 20: 230-235. 1936.

⁶ KADOW, K. J., and ANDERSON, H. W. BRITTLE ROOT OF HORSE RADISH IN ILLINOIS. U. S. Bur. Plant Indus., Plant Dis. Repr. 20: 288. 1936.

(3, Rept. 21; 31), dames rocket (*Hesperis matronalis* L.),⁷ and stock (*Matthiola incana* R. Br.) (12, 31, 38). The mosaic disease of water cress (*Nasturtium officinale* R. Br.) (3), was shown to be due to a strain of cucumber virus 1. The identity of many of the other virus diseases just mentioned, however, remains in doubt because of the lack of

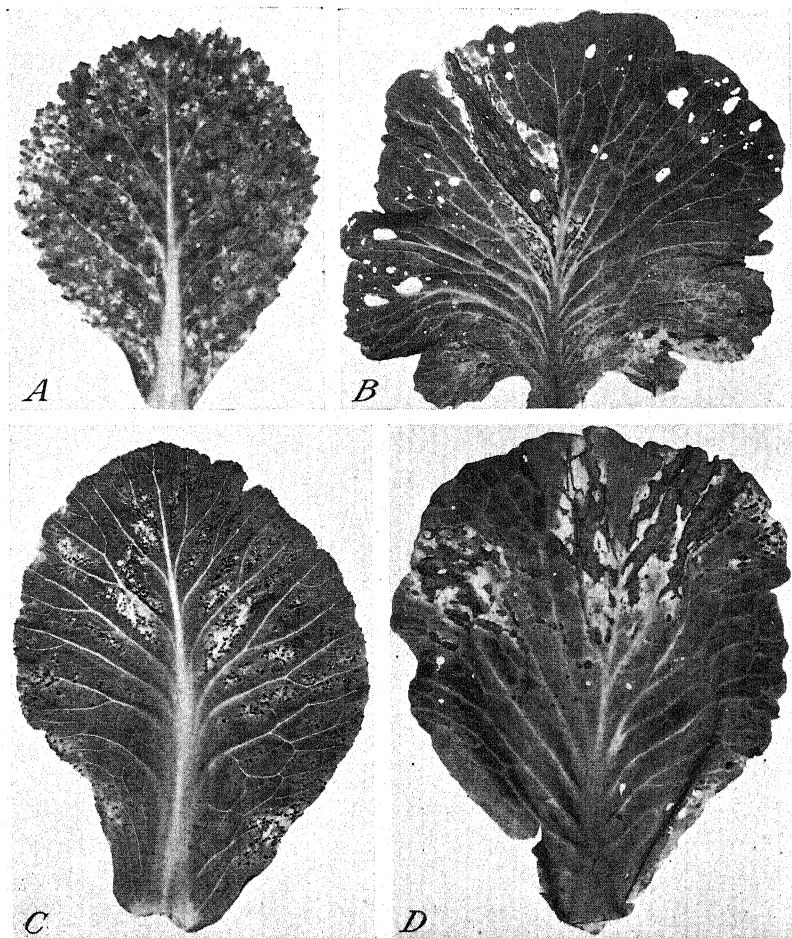


FIGURE 1.—A, Young infected leaf showing mottle and chlorosis; B, chlorosis followed by necrosis in a leaf in which infection has progressed for a longer interval; C and D, outer leaves of diseased mature cabbage showing chlorosis and bands of necrotic tissue. In C the necrosis is limited to an area extending from midrib to margin.

sufficient study of the virus concerned in each case. It is obvious that the time has come when the mere record of a mosaic disease of crucifers is of little value. A thorough analysis of symptomatology of the disease on various susceptibles and a study of the properties of the virus are essential for distinction.

⁷ See footnote 4.

SYMPTOMS OF MOSAIC DISEASE ON CABBAGE

The appearance of the mosaic disease as it occurs on cabbage in the greenhouse and under field conditions in southeastern Wisconsin will be considered first. Symptoms do not develop in the inoculated leaves but rather in those which become infected later through systemic spread of the virus. The first symptom on young systemically invaded leaves is a slight yellowing with a clearing of the veins beginning usually at the distal portion of the leaf. This is followed by mottling and more distinct vein clearing (fig. 1, *A*). The symptoms which

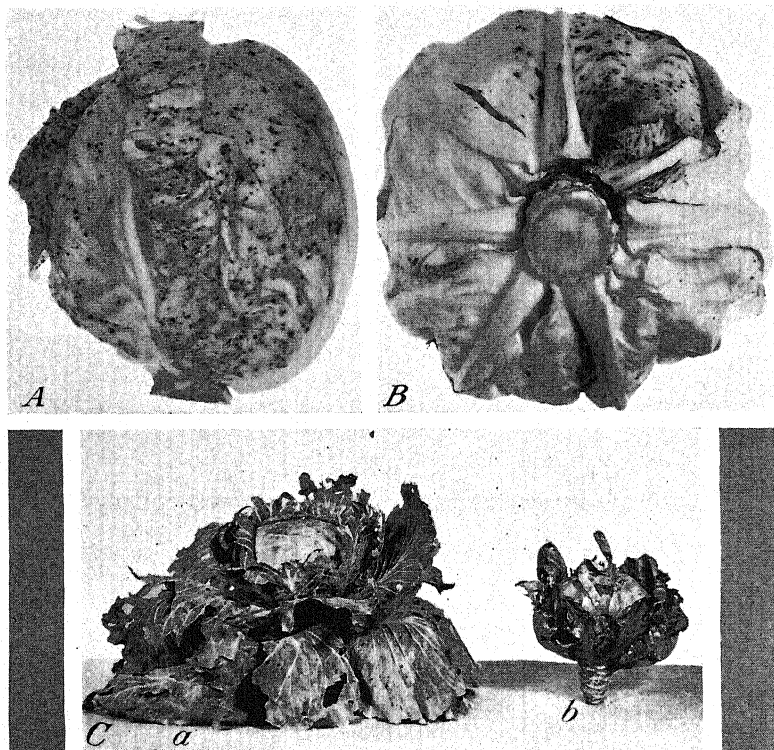


FIGURE 2.—*A*, Mature cabbage head infected with the virus showing internal necrosis of the parenchyma. *B*, Parting of leaves from core due to abscission-layer formation as a result of virus infection. *C*, *a*, uninoculated control; *b*, plant inoculated during the early part of the season, showing in comparison with the control the effect of progressive stunting and defoliation.

develop later vary considerably. They commonly consist of slight vein banding and savoying of the leaf lamina while the normal production of "leaf bloom" is retarded. The retardation of bloom produces a distinct off-type color in the bluer varieties, giving them a decidedly yellowish cast. As infected leaves become older, necrotic spots commonly occur on or along the veins and in the parenchyma (fig. 1, *B*). The spots on young inoculated plants are usually 1 to 3 mm. in diameter, sunken, and blue black in color. On the veinlets they may be linear, but on veins and midribs they are circular. The

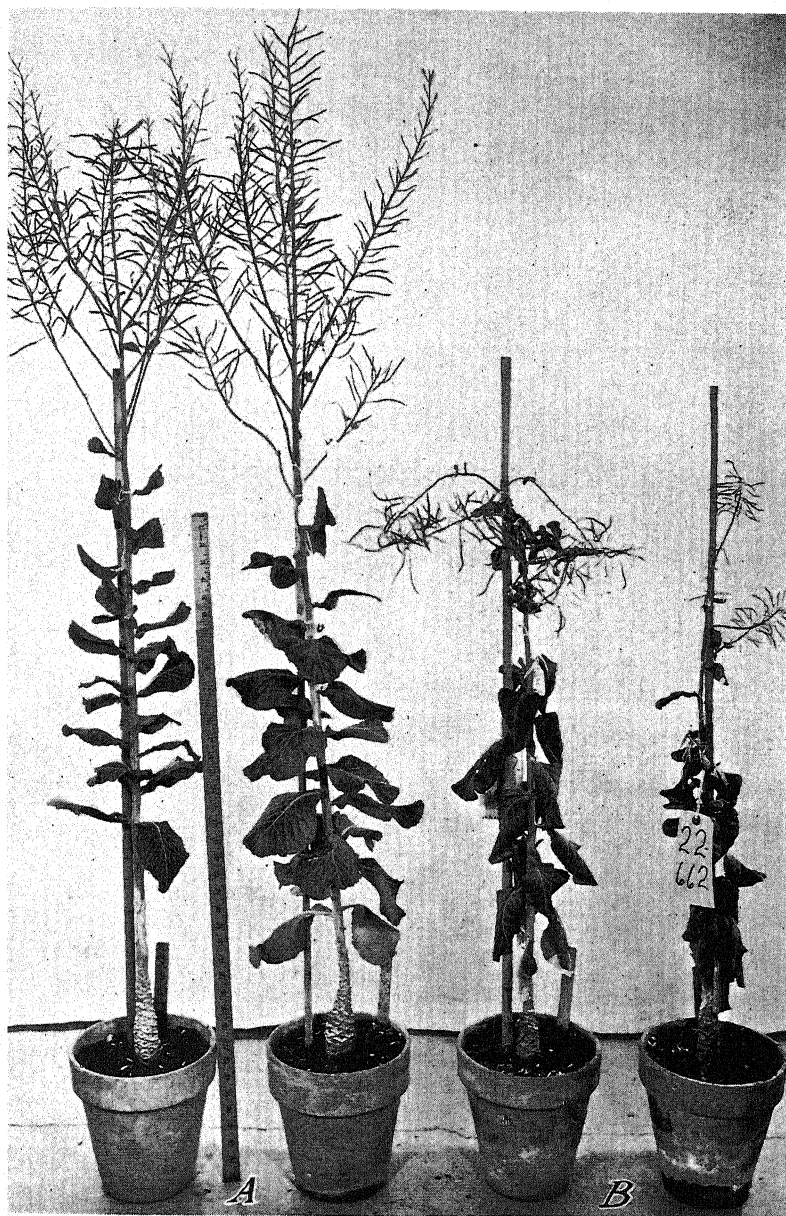


FIGURE 3.—*A*, Virus-free seed plants; *B*, advanced stage of virus symptoms on infected seed plants, consisting of declined vigor, defoliation, and impaired seed set.

savoying of the interveinal tissue, which is usually a deeper green than normal, is often followed by twisting or curling of the midrib.

In the field the symptoms may be masked very effectively, depending upon environment and possibly to some extent upon the variety. Warm midsummer weather is favorable to this disease, and in south-

eastern Wisconsin July and August are the months when it appears most conspicuously. Growth may be stunted without any sign of mottle or necrosis. Owing to leaf chlorosis and suppression of bloom, the color may appear yellower than normal. The outer leaves are inclined to be more erect and, being curved along the midrib, to clasp the head more tightly than is normal. The outer leaves show a variety of effects. Diffuse to prominent mottling may occur, sometimes followed by necrotic spots in the interveinal regions of the leaves.

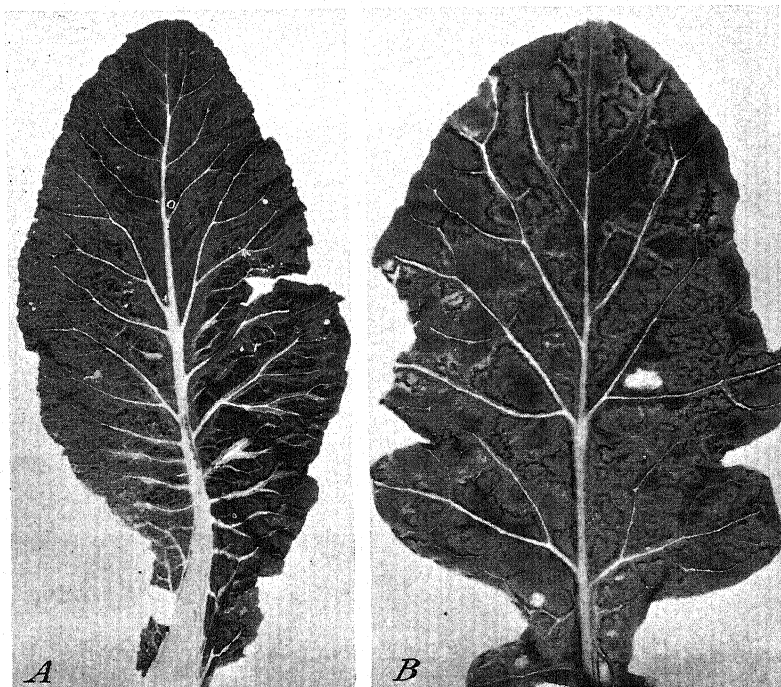


FIGURE 4.—A, Leaf of mature cauliflower infected with the virus, showing vein banding, mottle, chlorosis, distortion, and midrib twisting; B, chlorotic and necrotic mottle in the form of vein banding on sprouting broccoli.

Or, with little evidence of chlorosis, a necrosis may occur, either in spots or in continuous or interrupted bands which often extend to the edge of the leaf. These necrotic areas are brown, blue black, or purple, and as they grow older the tissue becomes dry and brittle (fig. 1, C and D). A common effect of systemic infection of cabbage is premature leaf drop. This may proceed whether or not other symptoms are conspicuous or even distinguishable. This condition results from premature formation of the abscission layer between the midrib or petiole and the main stem, and it is one of the most destructive phases of the disease (fig. 2, C).

Head necrosis also occurs (fig. 2, A, B), although it is not so frequent as the symptoms already described. It develops either as the head approaches maturity in the field or later in storage or transit. Sometimes it is confined to a few outer head leaves but it may be

scattered throughout the head. Commonly only a portion of a head leaf is affected from exterior to center. The necrosis is usually in the form of small, sunken, brown to black spots which are most numerous in the interveinal tissue where they may merge with one another to form larger spots. Black leaf speck may also result from suboxidation or low temperatures in storage, but this type is not associated with premature leaf drop. One common effect of the virus on the stored heads is the continuation of the untimely and premature formation of the abscission layer between petiole and main stem (fig. 2, *B*).



FIGURE 5.—*A*, Chinese cabbage inoculated with the cabbage mosaic virus, showing stunting, vein clearing, chlorosis, and leaf twisting, while the older leaves show necrosis; *B*, uninoculated control of the same age.

This effect may proceed up the entire length of the core, making the head worthless for market.

The masking of symptoms in cabbage plants is emphasized in holding them for seed production. It has been a common observation by the writers that normal healthy appearing plants selected for seed purposes often develop severe symptoms after the seed stem and branches have emerged in the greenhouse, or in the field the following season. Mottle of leaves, stems, and pods is common in such cases, followed by necrosis, drying, and premature dropping of the leaves. The vigor of such plants is often greatly reduced, and an increase in blasting of flower buds may occur (fig. 3). Necrosis of seed pods and impaired seed set have been often observed.

SYMPTOMS ON OTHER CRUCIFERS

The cabbage mosaic disease occurred in destructive form on cauliflower in southeastern Wisconsin in 1936, and the virus, when recovered, was found to be identical with that on cabbage in the same area. When young cauliflower plants are inoculated in the greenhouse the first symptoms are vein clearing, slight mottling, and yellowing of the younger leaves. The vein clearing gradually changes to vein banding, which consists of continuous, narrow, dark-green bands parallel with the midrib and veins but more conspicuous along the veins (fig. 4, A). Chlorotic bands along the veins may occur as well.

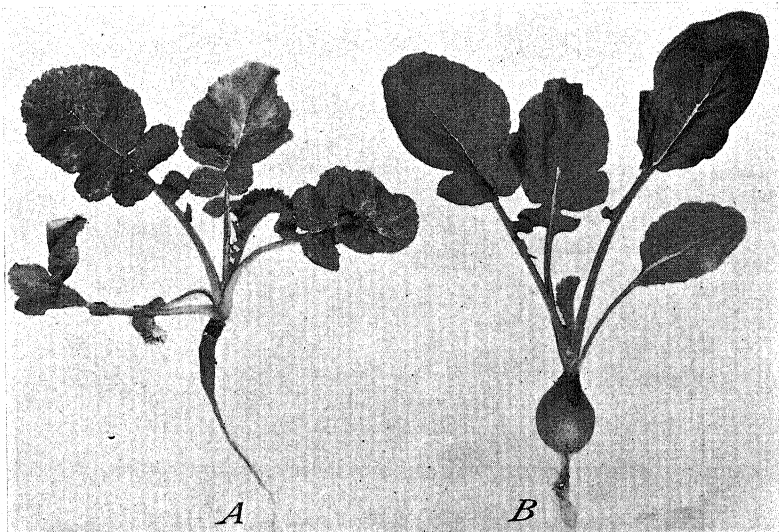


FIGURE 6.—A, Radish (Globe type) infected with the cabbage mosaic virus showing systemic infection, stunting, chlorosis, and retarded development of the fleshy hypocotyl; B, uninoculated control.

Mottle may consist of small spots or large areas. Necrosis appears later in the form of brown to blue-black linear lesions at the outer edges of the green or chlorotic bands along the veins. In the field vein banding and leaf distortion are particularly conspicuous as well as mottle and necrotic banding. Leaf distortion is much more conspicuous than on cabbage. The symptoms are often more severe on one side of the leaf than on the other and the resultant uneven growth causes much twisting of leaves and one-sided development of the plant as a whole.

In other subspecies of *Brassica oleracea*—kale, Brussels sprouts, kohlrabi, and sprouting broccoli—systemic vein clearing is again the first sign of infection, while conspicuous mottling, necrosis, leaf distortion, and general retardation of growth are conspicuous. Chlorotic mottle followed by necrotic bands parallel with the veins commonly develops on these hosts as on cauliflower (fig. 4, B).

Chinese cabbage is very susceptible to the cabbage virus. Vein clearing, chlorosis with some mottling, and necrosis are conspicuous. Leaf distortion is very common and pronounced general stunting of

the plant follows early infection under optimum environmental conditions (fig. 5).

Infected radish leaves develop vein clearing, mottle, slight chlorosis, and savoying. There is also some malformation and twisting. The plants are stunted and normal enlargement of the hypocotyl is retarded (fig. 6).

Symptoms on annual stock (*Matthiola incana* (L.) R. Br.) consist of vein clearing, chlorosis along the veins, mottling, and slight to severe stunting. Leaf malformation tends to be linear with some twisting and curling (fig. 7). Complete or sectional breaking of the flowers

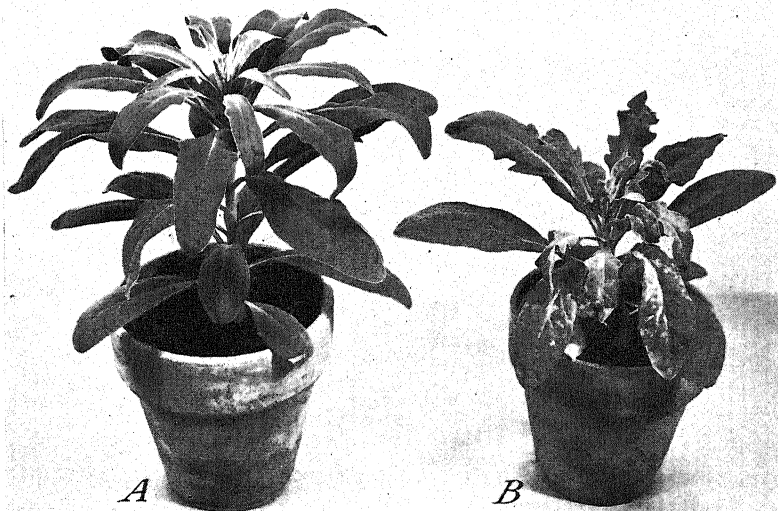


FIGURE 7.—A, Healthy *Matthiola incana* (annual stock), B, virus-infected plant showing stunting, vein clearing, and a limited amount of chlorosis.

in the form of streaks or bands results in distortion, with undersized and variegated petals (fig. 8).

Other cultivated crucifers found to be susceptible are listed later in the paper. The chief symptoms on all of these are varying degrees of systemic vein clearing, chlorosis, mottling, leaf distortion and stunting. The mottling is very conspicuous on dames violet (fig. 9).

TRANSMISSION STUDIES

METHODS AND MATERIALS

The virus used in these studies was obtained from cabbage grown in the yellows-resistance trial plot in Kenosha County, Wis., and has since been propagated on cabbage in the greenhouse at Madison, Wis. Frequent transfers were made to healthy cabbage to insure a constant supply of inoculum. The greenhouse temperature usually ranged from 22° to 25° C., and frequent fumigation for the control of insects was practiced. Artificial inoculations were made with extracted juice, and powdered carborundum was used regularly as an abrasive on cruciferous hosts (27), but was found to be unnecessary on many of the other susceptible hosts.

Healthy cabbage and turnip plants were employed for rearing colonies of the peach aphid (*Myzus persicae* (Sulz.)) and cabbage aphid (*Brevicoryne brassicae* (L.)). To increase vigor and to purify

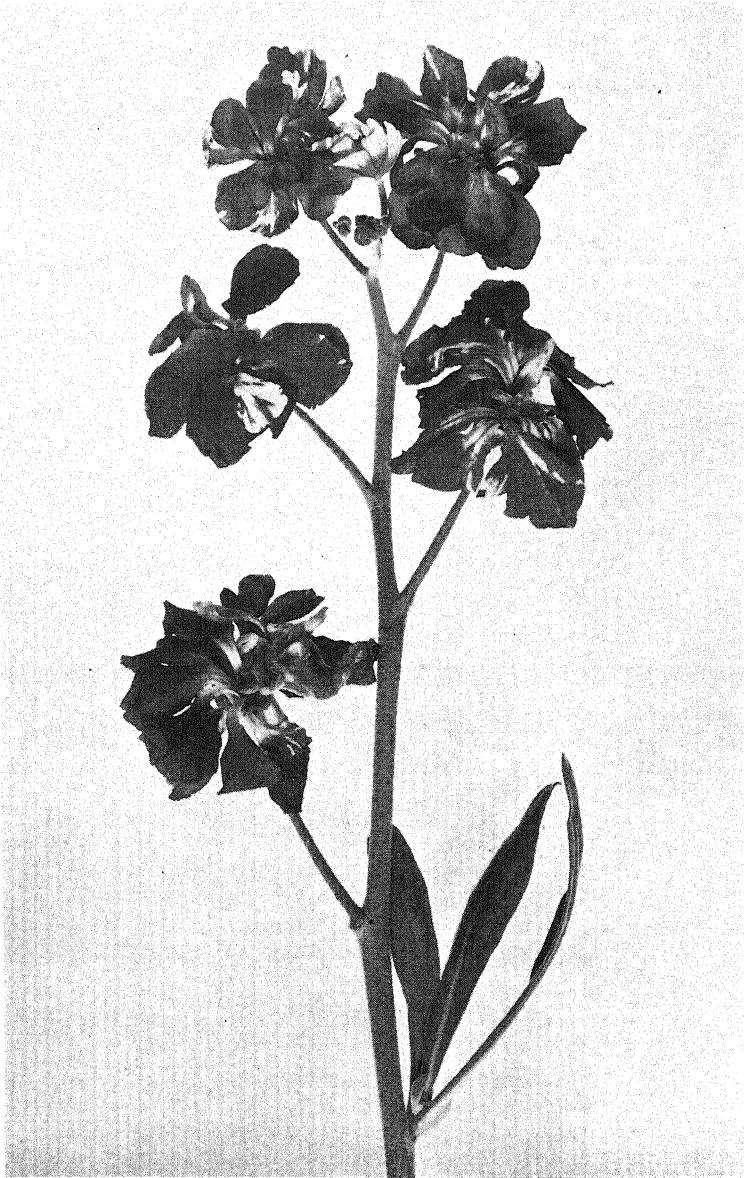


FIGURE 8.—“Breaking” of flowers in the terminal racemes of *Matthiola incana* infected by the cabbage mosaic virus; petals undersized and variegated.

the cultures, new colonies were increased by transferring winged adults. Small numbers of viruliferous aphids were transferred directly to test plants with a camel's-hair brush, avoiding contact between the trans-

fer brush and the test plants. When a large number was to be transferred, a leaf with many aphids was detached and placed on a small piece of paper and the paper was allowed to rest on the leaf of the healthy test plant until the viruliferous aphids migrated to the plant. Reinoculations from test plants were made to tobacco and cabbage to determine the presence or absence of the virus. All plants tested for susceptibility to the virus were checked in this manner. Sus-

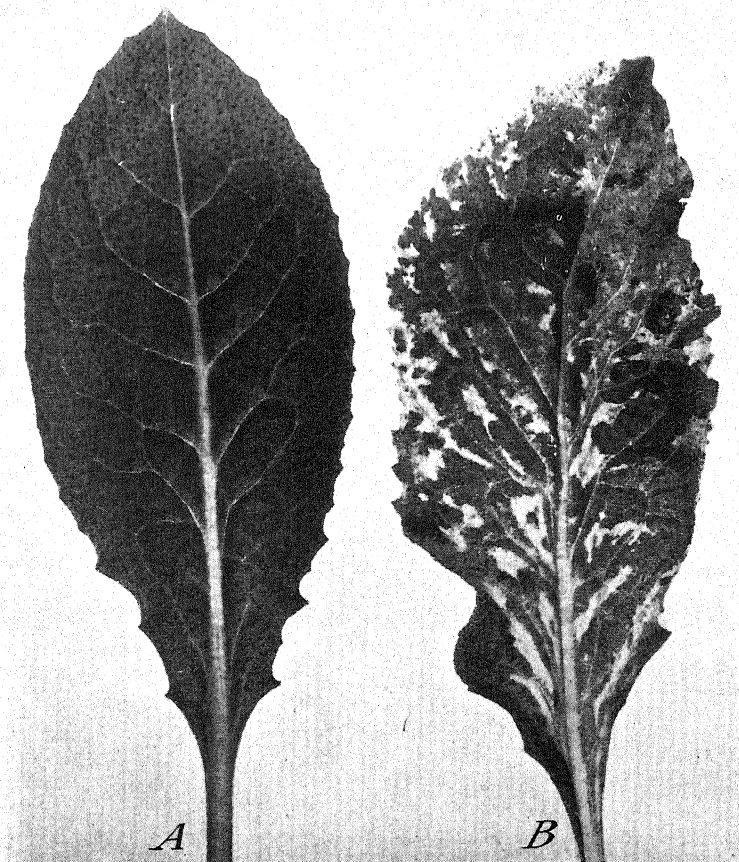


FIGURE 9.—Symptoms produced by the cabbage mosaic virus on leaves of dames violet: A, Leaf from uninoculated control; B, leaf from inoculated plant showing very conspicuous mottling.

ceptible species were repeatedly tested and compared with uninoculated controls. Details of unusual methods are described later.

CULTIVATED CRUCIFERS SUSCEPTIBLE TO THE VIRUS

All the cruciferous species tested were found capable of harboring the virus and of exhibiting symptoms when infected. The following list contains the forms tested; the symptoms on many of these have already been described.

Brassica oleracea viridis L. (kale—var. Dwarf Green Curled).

B. oleracea L. *gemmifera* DC. (Brussels sprouts—var. Long Island Improved).

- B. oleracea capitata* L. (cabbage—vars. Wisconsin Hollander, Wisconsin All Seasons, Jersey Queen, Marion Market, Golden Acre, Danish Ballhead, and Mammoth Rock Red).
B. oleracea botrytis L. (cauliflower—var. Snowball).
B. oleracea botrytis L. (broccoli—var. Green Sprouting).
B. oleracea gongylodes L. (kohlrabi—var. Early White Vienna).
B. napus L. (rape—var. Dwarf Essex).
B. campestris (L.) *napobrassica* DC. (rutabaga—vars. American Purple Top and White Russian).
B. rapa L. (turnip—vars. Purple Top White Globe and Snowball).
B. juncea (L.) Coss. (leaf mustard—var. Tender Green).
B. pe-tsai Bailey (Chinese cabbage—var. Chihli).
B. alba (L.) Boiss (white mustard).
Cheiranthus allionii Hort. (Siberian wallflower).
Malthiola incana R. Br. (stock—var. Dwarf Ten Weeks).
Hesperis matronalis L. (dames violet).
Lepidium sativum L. (garden cress—var. Extra Curled).
Raphanus sativus L. (radish—vars. French Breakfast and Crimson Giant).

WILD CRUCIFEROUS HOSTS

All cruciferous weeds inoculated were found to be susceptible. Dwarfing and slight mottling with some vein clearing are the usual symptoms. In the case of shepherds-purse, wild peppergrass, and hoary alyssum the early symptoms on fairly old leaves appear in 10 to 15 days after inoculation as chlorosis along the leaf margins, slight savoying, and mottle, followed eventually by necrosis and death (fig. 10). Young leaves become dwarfed and curled, but they persist and give the plants a stunted appearance. This dwarfed, rosette appearance is typical of the naturally infected plants of these weeds found in the field. The seedstalks and pods from such plants are usually malformed.

The following species were tested. In each case the habit of the plant in the upper Mississippi Valley is indicated (A=annual; WA=winter annual; B=biennial; P=perennial).

- Berteroa incana* (L.) DC. (hoary alyssum) A or P.
Capsella bursa-pastoris (L.) Medic. (shepherds-purse) A or WA.
Nestia paniculata (L.) Desv. (ball mustard) A or WA.
Radicula palustris (L.) Moench. (marsh cress) A or B.
Sisymbrium officinale (L.) Scop. (hedge mustard) A or WA.
Sisymbrium altissimum L. (tumblemustard) A or WA.
Lepidium virginicum L. (wild peppergrass) A or B.
Lepidium sativum L. (peppergrass) A or B.
Thlaspi arvense L. (pennycress) A or WA.
Brassica arvensis (L.) Ktze. (charlock) A.
Brassica nigra (L.) Koch (black mustard) A.
Brassica juncea (L.) Coss. (Indian mustard) A.

NONCRUCIFEROUS HOSTS

In contrast to hosts that show a mottle and chlorosis when systemically infected with the cabbage mosaic virus, Swiss chard (*Beta vulgaris cicla* L.) and sugar beet (*B. vulgaris* L.) develop an erratic localized type of systemic lesions (fig. 11). The first symptoms exhibited are numerous small dark local spots. These increase in size, the center changing to a brick red, and on coalescence, necrotic areas develop in the parenchyma, giving the effect of vein banding. In the older infected leaves severe necrosis develops first at the distal portion and progresses toward the base. The tissues gradually become dry and brittle and the leaf dies.

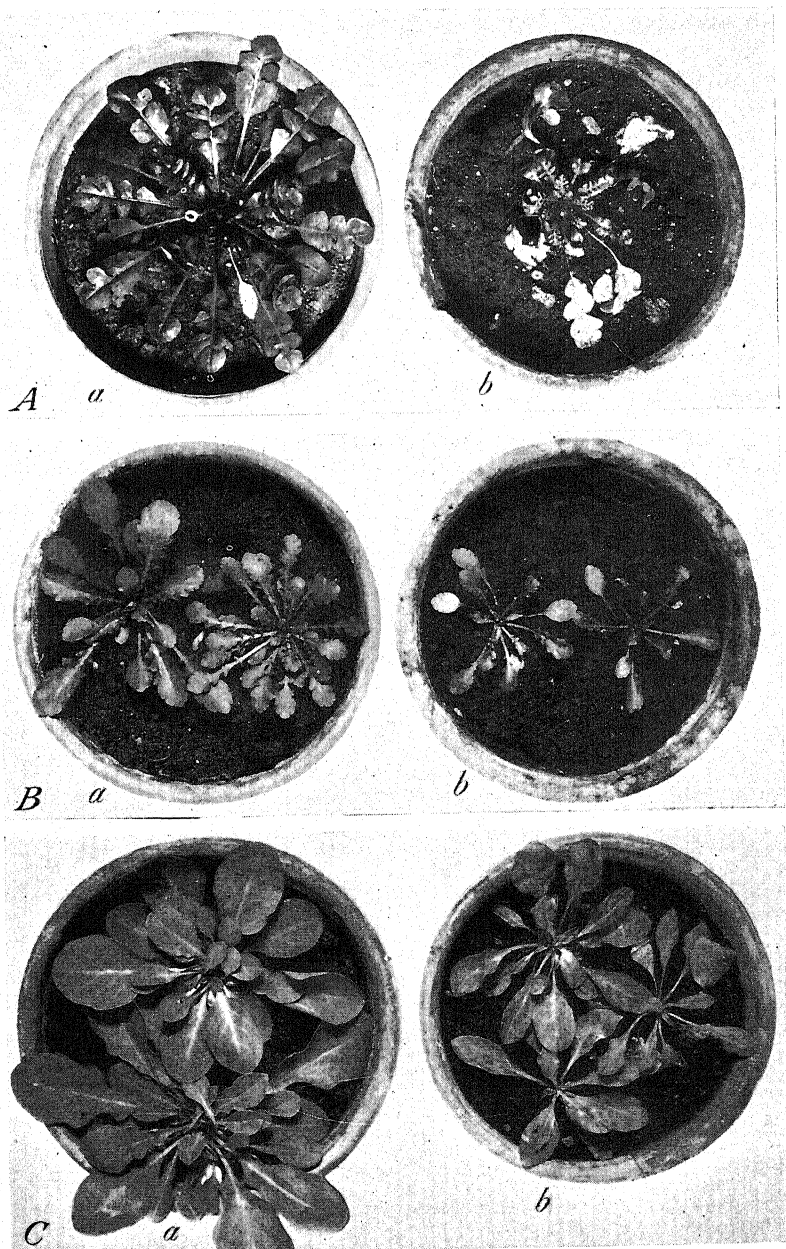


FIGURE 10.—Cruciferous weeds infected with the cabbage-mosaic virus: A, Shepherd's-purse; B, wild peppergrass; C, hoary alyssum. *a*, Uninoculated controls; *b*, inoculated plants.

Systemic infection in spinach (*Spinacia oleracea* L. var. Bloomsdale) appears as conspicuous vein clearing, well-marked progressive chlorosis, and some necrosis. Considerable malformation of the leaves and stunting are very evident (fig. 12, B, *b*).

Infected larkspur (*Delphinium ajacis* L.) develops mottle with slight necrosis at the tips of the leaves. Later bleaching and vein banding of the leaves is evident. The older infected leaves become dry and brittle and drop. The infected plants are usually very much stunted. The flowers show breaking in the form of white streaks or flecks. Systemic infection in petunia (*Petunia hybrida* Vilm. var. Rose King)

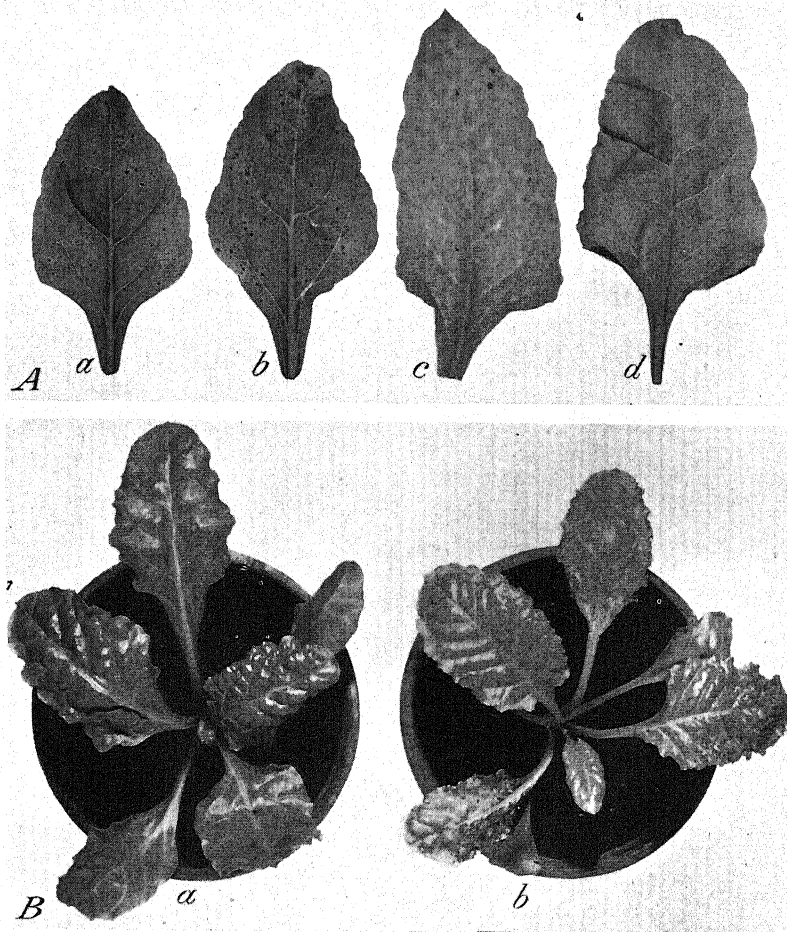


FIGURE 11.—A, Erratic type of systemic infection on leaves of sugar beet (a, b, c) infected with the cabbage-mosaic virus; uninoculated control (d). B, b, Infected plant of Swiss chard showing localized or spot type of systemic infection; a, uninoculated control.

results in a marked stunting, conspicuous mottle, and chlorosis without breaking of the flower (fig. 12, A). In calendula (*Calendula officinalis* L. var. Balls Orange) the first symptoms appear as clearing of the veins and mottle. This is followed by pronounced mottling and necrotic spotting. A twisting of the leaves and severe stunting are evident. Zinnia (*Zinnia elegans* Jacq. var. Orange and Gold) shows vein clearing followed by chlorosis and stunting.

REACTION OF SPECIES OF NICOTIANA

Infection of cruciferous and noncruciferous hosts described above was definitely systemic. In the species of *Nicotiana* tested infection was either localized, erratic (i. e., inoculation resulting in local lesions followed by systemic infection), or systemic (fig. 13). In tobacco (variety Connecticut Havana No. 38), *N. tabacum calyciflora* L., *N.*

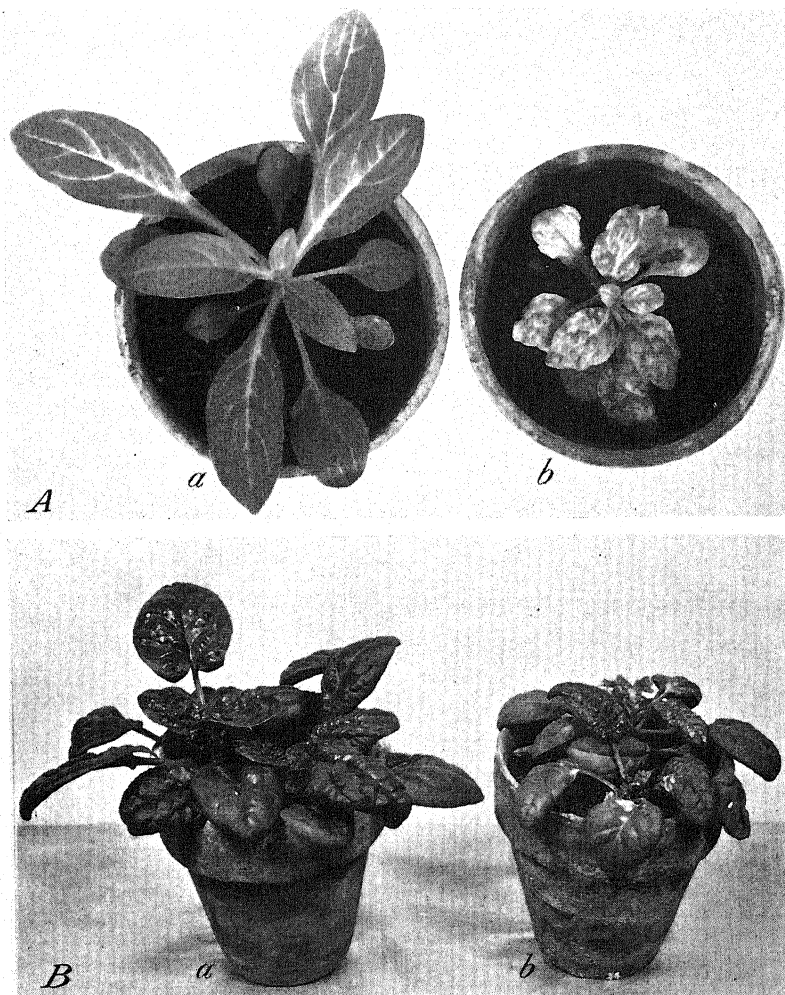


FIGURE 12.—A, a, Uninoculated petunia; b, petunia infected by the cabbage-mosaic virus, showing pronounced vein clearing, chlorosis, and stunting. B, a, Uninoculated spinach; b, virus-infected spinach plants showing stunting and conspicuous chlorosis.

syvestris Speg., and in the F_1 hybrid of *N. tabacum* \times *N. glutinosa*, small individual necrotic lesions appear on the inoculated leaves in from 3 to 4 days. The necrotic area enlarges rapidly up to 3 cm. or more in diameter, usually showing a brick-red center with concentric rings and a darker band at the edge. The older lesions become dry

and die out, and as they coalesce a large part of the leaf becomes involved. No systemic invasion occurs. *N. sylvestris* differed from the rest in that the local lesions did not appear on the inoculated leaves until 12 to 15 days after inoculation.

In *Nicotiana glutinosa* a faint halo type of chlorosis appears in from 12 to 15 days on systemically infected leaves, which is followed by a

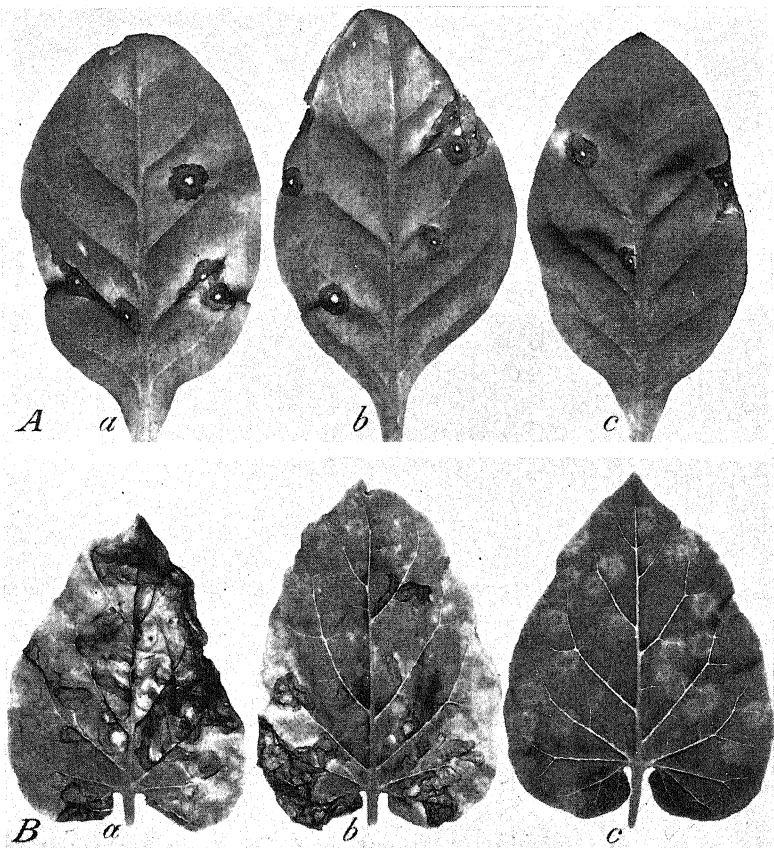


FIGURE 13.—A, *a-c*, Older local lesions on the F_1 hybrid of *Nicotiana tabacum* \times *N. glutinosa* produced by transferring *Myzus persicae* from cabbage infected with the mosaic virus. B, Infected leaves of *N. glutinosa*: *a* and *b*, Older chlorotic lesions and necrosis; *c*, early halo type of chlorotic spots that form on systemically infected leaves to which the virus has spread from lower inoculated leaves.

more conspicuous yellowing with slight necrosis. The necrosis gradually involves the entire leaf but the virus is not fatal, for symptoms continue without preventing flowering and seed setting (fig. 13, B). In *N. rustica* L. no primary symptoms appear on the inoculated leaves. Secondary symptoms appear in about 15 days, consisting of irregular, diffused chlorosis and definitely marked progressive mottling of light and dark-green areas. Leaf distortion may follow in the form of one-sided infection and twisting (fig. 14, A, *a, b*).

In *Nicotiana repanda*, Sims primary local lesions appear in 3 to 4 days with dark centers and concentric rings. They enlarge and coalesce to form an irregular necrotic pattern, the inoculated leaves often becoming completely necrotic and dry. Early secondary symptoms consist of mild local chlorosis followed successively by mottling and necrosis. The plants usually are very much stunted. In *N. quadrivalvis*

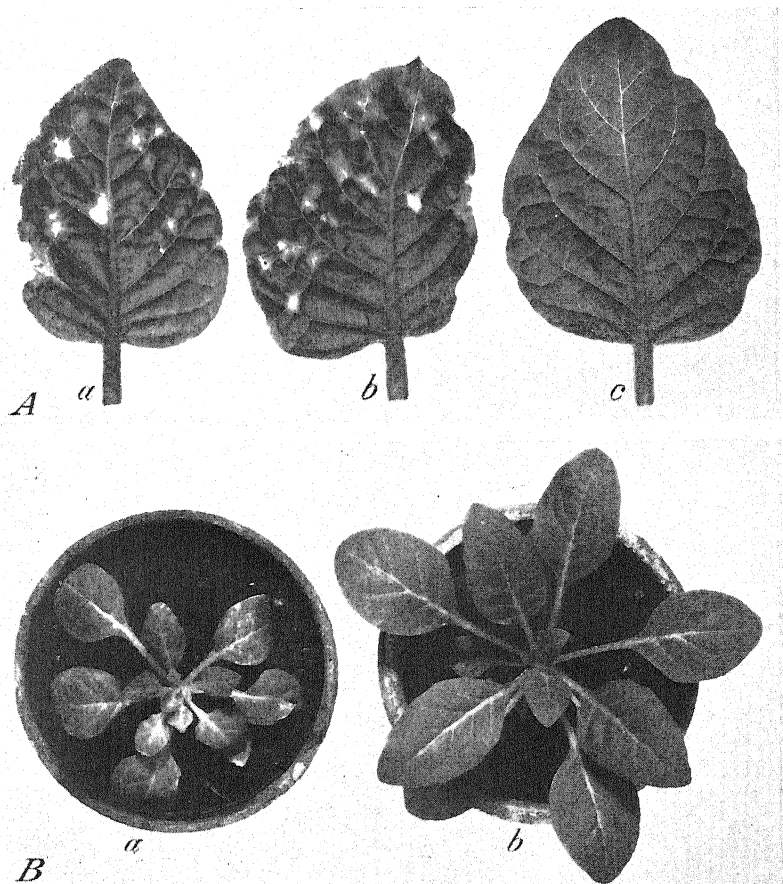


FIGURE 14.—A, Symptoms of systemic infection of *Nicotiana rustica* by the cabbage-mosaic virus are chlorotic spotting and distortion (a, b); Healthy control (c). B, a, Stunting, vein clearing, chlorosis, and leaf malformation of *N. multivalvis* infected with the cabbage-mosaic virus; b, uninoculated control.

Pursh primary symptoms were not observed on the inoculated leaves. Vein clearing and yellowing of the younger leaves occurred in from 14 to 16 days, followed by stunting of the growing point. Leaf distortion in the form of twisting and narrowing appears with progressive chlorosis and savoying. No necrosis develops and the symptoms continue to appear until flowering. Chlorosis, vein clearing, and stunting of the younger leaves are evident on *N. multivalvis* Lindl. in about 15 days, with no primary symptoms on the inoculated leaves. The chlorotic patterns gradually become necrotic in older plants (fig. 14, B, a).

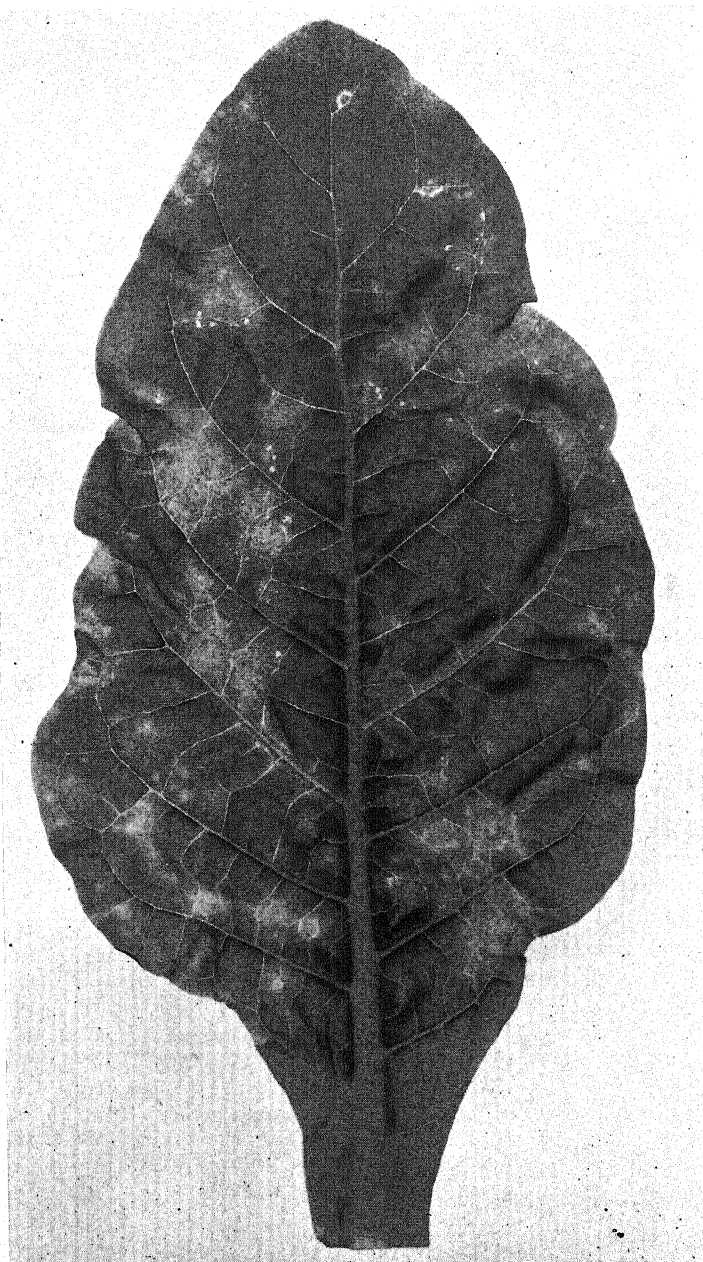


FIGURE 15.—Leaf symptoms produced by systemic infection of the cabbage-mosaic virus on *Nicotiana langsdorffii*, showing chlorotic mottling and distortion of the intercostal area.

Systemic infection appears in the form of conspicuous chlorosis on *N. langsdorffii* Schrank. Well-marked mottling of light- and dark-green areas with some leaf distortion follows (fig. 15). In *N. bigelovii* (Torr.)

S. Watts the virus produces pronounced, systemic infection in the form of conspicuous stunting and chlorosis 15 days after inoculation.

SPECIES NOT INFECTED BY THE VIRUS

Attempts to transmit the cabbage-mosaic virus to the following species were unsuccessful:

Yellow dock (*Rumex crispus* L.); buckwheat (*Fagopyrum esculentum* Moench), var. Silver Hull; pigweed (*Chenopodium album* L.); broad-bean (*Vicia faba* L.); nasturtium (*Tropaeolum majus* L.); var. Golden Gleam; pansy (*Viola tricolor* L.), var. Black King; tomato (*Lycopersicon esculentum* Mill.), var. Globe; red currant tomato (*L. pimpinellifolium* Dunal); potato (*Solanum tuberosum* L.), vars. Irish Cobbler and Rural New Yorker; nightshade (*S. nigrum* L.); eggplant (*S. melongena* L.), var. Black Beauty; buffalo-bur (*S. rostratum* Dunal); *Nicotiana sanderae* Sander; *N. longiflora* Cav.; *N. nudicaulis* S. Wats.; snapdragon (*Antirrhinum orontium* L.), var. Rust Proof; muskmelon (*Cucumis melo* L.), var. Milwaukee Market; cucumber (*C. sativus* L.); var. Chicago Pickling; Watermelon (*Citrullus vulgaris* Schrad.), var. Stone Mountain; dandelion (*Taraxacum officinale* Weber); head lettuce (*Lactuca sativa* L. *capitata* Hort.), var. Iceberg; and China-aster (*Callistephus chinensis* (L.) Nees), var. Giant Blue (Wilt resistant).

INSECT TRANSMISSION

The species of insects found on cabbage in the field, viz, the cabbage aphid (*Brevicoryne brassicae*), green peach aphid (*Myzus persicae* Sulz.), and the imported cabbage worm (*Pieris rapae* L.) were studied as possible vectors. In view of possible variations in the concentration of the virus in leaves of different ages on the same plant, viruliferous aphids from leaves of corresponding ages were used in all transmission studies. Cabbage (var. Jersey Queen) and shepherds-purse were used as test plants. In tests with the cabbage aphid it was found that the feeding time required for nonviruliferous apterous forms to acquire the active principle was one-half hour and transmission of the virus was accomplished in a period of equal length. When the apterous non-viruliferous peach aphids were tested, the time to acquire the virus was slightly longer, although transmission of the virus to healthy test plants resulted after a feeding time of one-half hour. The short feeding period on both diseased and healthy test plant indicate quite definitely that a long incubation period of the virus within the two vectors does not take place. It was also found that viruliferous cabbage and peach aphids are capable of infecting four consecutive healthy test plants without intermittent feeding on the source of inoculum. A feeding period of 1 hour was allowed for each successive test plant. The possibility of reinfection of the aphids with accumulated virus from the test plant does not seem likely because of the short feeding period on consecutive test plants. The alate forms of both species were also shown to be capable of serving as vectors.

The cabbage worm was found to be effective in transmission of the cabbage mosaic virus in greenhouse tests. A small number, usually three, viruliferous cabbage worms, having previously fed on diseased plants for 24 hours, were transferred directly to the test plants and allowed to feed for as much as 4 hours and not more than 6 hours since longer feeding periods completely defoliated the test plants. In four series of 5 plants each, it was found that 16 of the 20 test plants produced typical symptoms.

TEMPERATURE RELATIONS OF THE DISEASE

The relation of temperature to the disease was studied under controlled conditions in the greenhouse. Healthy cabbage plants were artificially inoculated and an equal number (20) placed in each of a series of greenhouses which were kept as uniformly as possible at 16°, 20°, 24°, and 28° C., respectively. An equal number of checks was included. Two such experiments were conducted with similar results. At 16° a very slight mottling developed, visible with transmitted light only, and there was no vein clearing. Otherwise it was not possible to distinguish between inoculated and healthy plants. Slight symptoms occurred at 20° and a few plants showed vein clearing. At 24°, vein clearing and a distinct increase in mottling occurred with some necrosis on most plants. Pronounced symptoms occurred at 28°, although an increase in the chlorotic type of mottle developed. When diseased plants grown at the higher temperatures (24° or 28°) were removed to the cooler greenhouse (16°), new foliage of normal appearance developed. These series showed conclusively that the disease is expressed more rapidly and severely at high air temperatures, a result which is in full accord with the observations made upon the epidemiology of the disease in the field.

PROPERTIES OF THE VIRUS

In the study of the properties of the cabbage-mosaic virus, expressed juice of diseased cabbage plants was treated and then used to inoculate tobacco as the test plant. The average number of local lesions on one leaf of each of 10 plants in three trials each was used as the criterion for comparison (table 1). The longevity in vitro at about 20°-22° C. was found to be between 24 and 48 hours. The virus has an inactivation temperature at or near 55° for a 10-minute treatment and a dilution tolerance of about 1 to 1,000. The virus is readily inactivated by drying.

TABLE 1.—*Properties of the cabbage-mosaic virus as determined by local lesions formed on inoculation to tobacco*

[10 plants inoculated in each of 3 trials]

Longevity in vitro		Thermal death-point		Tolerance to dilution		Longevity in vitro		Thermal death-point		Tolerance to dilution	
Period of exposure at 20°-22° C. (hours)	Average number of lesions on 30 leaves	Temperature of the 10-minute exposure	Average number of lesions on 30 leaves	Dilution	Average number of lesions on 30 leaves	Period of exposure at 20°-22° C. (hours)	Average number of lesions on 30 leaves	Temperature of the 10-minute exposure	Average number of lesions on 30 leaves	Dilution	Average number of lesions on 30 leaves
		°C.						°C.			
0.....	126	±20	130	0	141	48.....	9	54	3	1:1,000	1
12.....	92	52	121	1:10	81	72.....	0	55	0	1:2,000	0
24.....	24	53	12	1:100	11	96.....	0	56	0	1:3,000	0

In the study of virus diseases of cruciferous crops, reports of property studies have been given in only a few cases. A summary of the data reported are shown in table 2. Because of the uncertainty still existing concerning the number and identity of the viruses reported on cruciferous hosts, it seems well to indicate briefly the significant differ-

ence between the viruses mentioned in the table and the one described in this paper. The cabbage-mosaic virus reported herewith, when compared as to properties and host reaction with the mosaic on turnip of Hoggan and Johnson (16), appears to be similar if not identical. The cauliflower virus described by Tompkins (39) differs in the absence of infection on any species of *Nicotiana*, in the masking of symptoms at high temperatures (above 20° C.), and in each of the properties listed in table 2. The black-ring virus of cabbage differs from the cabbage mosaic virus in greater longevity in vitro, in a higher inactivation point, and in its low temperature optimum. The Chinese cabbage mosaic virus of Tompkins and Thomas (42) differs from the virus described herein in all of the properties studied and in the fact that it became systemic only in cruciferous hosts. The turnip mosaic virus of Tompkins (40) did not become systemic in cabbage.

TABLE 2.—Comparison of properties of crucifer viruses as determined by different investigators

Authority	Host	Virus disease	Longevity in vitro	Inactivation temperature (10 minutes)	Dilution tolerance
Hoggan and Johnson 1935 (16)...	Turnip.....	Mosaic.....	24-48 hours at 22° C.	54.....°C.	1-1,000
Tompkins 1937 (39).....	Cauliflower.....	do.....	14-15 days at 22° C.	75.....	1-2,000
Tompkins et al. 1937 (41).....	Cabbage.....	Black ring.....	72 hours at 22° C.	59.....	1-1,000
Tompkins and Thomas 1938 (42).....	Chinese cabbage.....	Mosaic.....	72-96 hours at 22° C.	Between 73 and 75.	1-5,000
Tompkins 1938 (40).....	Turnip.....	do.....	2-3 days at 22° C.	Between 60 and 63.	1-3,000
Larson and Walker.....	Cabbage.....	do.....	24-48 hours at 22° C.	55.....	1-1,000

RELATION OF THE VIRUS TO CABBAGE SEED

In 1936 mosaic was severe in the yellows-resistant cabbage plots in southeastern Wisconsin. There were some indications that infection occurred in the seedbed from which the plants were taken. This bed was in a secluded spot where crucifers had not been grown for many years. A large number of cabbage seed lots from a great many sources were included in this seedbed, and it is possible that the virus may have been introduced with one or more lots of seed.

During the winter of 1936-37 numerous diseased cabbage seed plants were grown in a cool greenhouse at 16°-20° C. for seed pod and seed formation. When extracted juice from young whole seed pods was used as inoculum all test plants developed virus symptoms. When only the young immature seeds (milk stage) were used as a source, the extract also proved to be infectious. Inoculations made with the extract from mature seed pods and with that from the mature seeds resulted in no infection of the test plants.

Mature seeds obtained from severely infected seed plants were used in seed-transmission trials in the greenhouse. A total of 5 plantings was made. All possible precautions were taken to prevent contamination from other sources. From a total of 1,764 plants, 26 were suspected of being diseased upon examination at the transplanting stage. These were reset for observation and it was found that 2 of the young plants showed definite mosaic symptoms and the juice extracted proved to be infectious. During the course of this experi-

ment between 3,000 and 4,000 plants were grown from seed from known healthy seed plants, in the same greenhouse, without any sign of disease at the transplanting stage.

While the data secured are not sufficient to serve as a basis for final deductions, two tentative conclusions seem to be clear. (1) The virus systemically invades the flower parts and the young pods and seeds, but if present in mature seeds it is there in such a state as not to be readily extracted. (2) The virus, if seed-transmitted, is not so perpetuated in a large percentage of cases even when seed is collected from very severely diseased pods. In spite of the very small number of cases of seed transmission, the growing of plants in crowded seedbeds and the effective means of spread by aphids combine to leave this means of virus introduction and dissemination a highly important one unless even rare seed transmission can be disproved with absolute certainty.

OVERWINTERING OF THE VIRUS

Since all cruciferous weeds found in Wisconsin that were tested proved to be susceptible, it was thought that the winter annuals and perennials that become infected in the latter part of the growing season as a result of migration of viruliferous cabbage or peach aphids might serve as an overwintering source of inoculum. A large number of cruciferous and noncruciferous weeds were collected in the early spring of 1937 before seedbed planting time. These weeds were growing on the borders of commercial cabbage and cauliflower fields that were heavily infected with the virus during the growing season of 1936. Many of the weeds collected were dwarfed and rosetted and their leaves were chlorotic and necrotic. Artificial and aphid (*M. persicae*) inoculations to young cabbage and shepherds-purse plants resulted in the recovery of the cabbage mosaic virus from only two cruciferous weeds, shepherds-purse and pennycress. None of the noncruciferous weeds yielded the virus. These two weeds are very common winter annuals in southern Wisconsin and may well become an important overwintering source of the virus or strains of the virus from which aphids may transmit to seedbeds and early transplanted fields in the spring.

RELATION OF CABBAGE VARIETIES TO THE DISEASE

In the course of field observations during the widespread occurrence of this disease in southeastern Wisconsin in 1935 and 1936 all the varieties exposed were infected. It was noted, however, that although general infection did occur some varieties were more severely damaged than others. Marion Market seemed to be most generally and severely affected, whereas Globe in adjacent plantings was less seriously damaged even though it sustained as high a percentage of infected plants. Wisconsin All Season seemed to produce quite successfully in spite of general virus infection.

Some preliminary results were secured at Madison, Wis., in 1936 in a comparison of inoculated and uninoculated plants of several standard varieties, all but one of which (Penn State Ballhead) had been selected for resistance to yellows (*Fusarium conglutinans* Wr.). One row of each variety was inoculated artificially with juice extracted from diseased plants, soon after the plants had recovered from trans-

planting. Thorough application of insecticides to control insect vectors was practiced throughout the growing season. At harvest weights of heads were taken from 10 inoculated and 10 uninoculated plants of each variety. The results are given in table 3.

TABLE 3.—*Comparative yields of healthy cabbage plants of different varieties and of plants inoculated with the cabbage-mosaic virus soon after transplanting*

Variety	Average weight of heads		Decrease of weight in diseased plants	
	Healthy	Diseased		
	Ounces	Ounces	Ounces	Percent
Penn State Ballhead.....	54.0	27.8	26.2	48.5
Marion Market.....	76.1	46.2	29.9	39.3
Wisconsin Ballhead.....	55.8	46.2	9.6	17.2
Wisconsin Hollander.....	49.2	41.3	7.9	16.1
Globe.....	63.0	53.3	9.7	15.4
Wisconsin All Seasons.....	71.5	63.7	7.8	10.9

These trials are too limited to furnish sufficient data upon which to base an evaluation of varieties. They do confirm, however, the field observation that Marion Market is more readily damaged than Globe and Wisconsin All Seasons. In this trial Penn State Ballhead appeared to be much more susceptible than the yellows-resistant varieties of the same type, Wisconsin Hollander and Wisconsin Ballhead.

DISCUSSION

It is obvious from the results of this investigation and that of other workers that a number of strains of virus affect the crucifers. Some of these appear to be confined to this family while others have a much wider host range. Some crucifers such as turnip and Chinese cabbage are very susceptible to all strains. On the other hand, some economic crucifers have a sufficiently differential reaction to aid somewhat in diagnosis.

The cabbage mosaic virus described in this paper seems to differ in several respects from crucifer viruses reported elsewhere. It is extremely virulent on cabbage in the Middle West, where its optimum temperature prevails at a time when the cabbage crop is usually just past the transplanting stage. Common cruciferous winter annuals in this area which become infected in late summer are capable of carrying the virus over winter readily. The cabbage aphid, which is commonly present, is an effective vector of the virus throughout the spring and summer. The customary procedure of growing plants in crowded seedbeds creates a situation especially favorable for extensive dissemination.

The recent sudden appearance of cabbage mosaic in destructive form cannot be explained with entire satisfaction. It has undoubtedly been present to a minor degree for many years. Symptoms are readily masked under a cool environment and many aspects of the foliage symptoms have undoubtedly been confused with those of other diseases.

Further study of the variation of this and other crucifer viruses is needed. In view of the findings in other virus groups it is reasonable to expect variations in respect to host range, properties, and virulence in this group. The extent of such variability will in some measure

determine the eventual severity of the disease and it will also have an important bearing on the progress that may be expected in the development of resistant varieties.

SUMMARY

The mosaic disease of cabbage discussed in this paper is one that has been extremely destructive to the crop in southeastern Wisconsin.

The symptoms brought about on cabbage and many cruciferous and noncruciferous hosts are described.

All cruciferous hosts, cultivated and wild, which have been tested were found to be susceptible.

Among noncruciferous hosts are three important crop plants, Swiss chard, sugar beet, and spinach, and three ornamentals, larkspur, petunia, and zinnia.

The reaction of several species of *Nicotiana* is given because of the importance of these facts in distinguishing the virus from others.

A list of plants that did not become infected upon inoculation is given.

Three insects found commonly in commercial cabbage fields, *Myzus persicae* (peach aphid), *Brevicoryne brassicae* (cabbage aphid) and *Pieris rapae* (cabbage worm) are vectors. *B. brassicae* picked up the virus from diseased cabbage plants in a feeding period of one-half hour and viruliferous aphids infected healthy test plants in a feeding time of one-half hour. *M. persicae* required 1 hour to secure the virus, although the feeding time for infecting healthy plants was the same as for the cabbage aphid. Both alate and apterous forms of the two species of aphids are vectors.

Environmental conditions play an important part in the destructiveness of the disease and expression of plant symptoms. The disease is most severe at temperatures of 24° to 28° C., within which range stunting and necrosis occur. New foliage appears symptomless when infected plants are held at 16° C. or lower.

The virus is transmitted mechanically with the aid of carborundum as an abrasive. It remains infectious in vitro for 24 to 48 hours at 20°–22° C. The tolerance to dilution is about 1 to 1,000; the virus is inactivated when held at about 55° C. for 10 minutes. Drying inactivates the virus.

The virus has been recovered from young seed pods and immature seeds of cabbage but not from mature pods or seeds. Seed-transmission has not been finally proved.

It has been definitely demonstrated that overwintering cruciferous weeds are an important source of inoculum in southeastern Wisconsin.

Indications from preliminary trials confirm field observation that certain cabbage varieties are more tolerant to the disease than others.

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VARIATION AND CORRELATION IN BUD MUTANTS OF THE MONTMORENCY CHERRY¹

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INTRODUCTION

A bud mutant differs so radically in one or more respects from the parent form as to give rise to the question whether if propagated, it will be less stable in some, or even all, its characters, than similar but normal structures from the same parental source. Should the mutant show a tendency toward greater variation in one or more of its aspects, the further question arises as to whether this variation is also more heterogeneous. That is, does a lower degree of correlation obtain for this greater variability? If so, an unusual potentiality for further mutation might reasonably be expected.

MATERIALS AND METHODS

Intraclass correlations² were employed as a means of studying variation in mutants of the Montmorency cherry (*Prunus cerasus* L.)

The trees available for measurement were six in number, standing adjacent in a row, on the same type of soil, at the Graham Horticultural Experiment Station, Grand Rapids, Mich. They originated from a limb mutation on a tree in an orchard near South Haven, Mich. This limb, kept under observation for several years, in contrast to the rest of the tree, formed no fruit buds and was always barren. Buds were taken from it in the summer of 1925 and propagated in the nursery. The resultant trees were set in the orchard in 1929. Upon coming into bearing, they displayed the following characteristics, which remained the same in succeeding years and are so at the present time:

Tree 1—Barren, except two main limbs that have reverted to normal fruit-bud formation and productivity.

Tree 2—Wholly barren.

Tree 3—Barren, except one main limb with reduced but definite and regular fruitfulness.

Tree 4—Like tree 1, except that it has only a single fruitful limb.

Tree 5—Wholly barren.

Tree 6—Wholly normal in bearing.

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² FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 5, rev. and enl., 319 pp., illus. Edinburgh and London, 1934.

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Of these six trees, four—Nos. 1, 4, 5, and 6—were used in the present study. These four provided two mixed specimens, one specimen entirely mutational, and one completely normal.

The character selected for measurement was leaf area (area of spur leaves), a character in which the range of variation is both wide and conspicuous.

The leaves were measured in situ. The length from base to apex, in millimeters, was converted into area by the use of a factor previously determined and established on the basis of printed and planed areas for excised leaves. Regularity of shape in the leaf of the Montmorency cherry permits such a procedure.

The data was gathered in midsummer, 1935. Measurement was restricted to nonbearing spur leaves of lateral branches on the wood of the year 1933. An additional set of measurements was secured from the 1934 wood on trees 5 and 6. Individual leaf areas were added to secure the total area per spur. Hence, in the logic and arrangement of intraclass correlation, each lateral branch, with its several spurs as fraternities, constitutes a general group.

PRESENTATION OF DATA

The results of the computations are shown in table 1.

TABLE 1.—*Intraclass coefficients for leaf areas*

Tree No.	Location of leaves	Pairings	Intraclass coefficients	z^1 values	σz^2	$z/\sigma z$
		<i>Number</i>				
1.....	(Barren part.....	1,560	0.1534	0.51119	0.1858	2.8
	(Fruitful part.....	1,008	.0794	.23910	.1827	1.3
4.....	(Barren part.....	896	.1769	.49240	.1955	2.5
	(Fruitful part.....	896	.1498	.42960	.1955	2.2
5.....	Barren tree.....	780	.2781	.59410	.1544	3.9
6.....	Fruitful tree.....	690	.1284	.30634	.1645	1.9
5.....	1934 wood.....	990	.1181	.41534	.2346	1.8
6.....	do.....	810	.0874	.33191	.2621	1.3

$$^1 z = \frac{1}{2} \log_e \frac{1+(k-1)r}{1-r}$$

$$^2 \sigma z = \sqrt{\frac{1}{2} \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}, \text{ with } n_1 = n' - 1, n_2 = (k-1)n'$$

The intraclass coefficients shown in table 1 are all positive. That for the barren part is apparently higher in each comparison. However, only three of the eight reach the level of probable significance. These three pertain to the barren parts of the trees. No one of the differences in the four comparisons of z is significant. The data, obviously inadequate as to number of observations, yield the suggestion, though not the full certainty, of real correlations in leaf area, those for the mutant forms being higher than those for the normal.

Incidentally, some information on variability as such, obtained by Fisher's method, is given in table 2.

TABLE 2.—Mean squares for variance in leaf areas

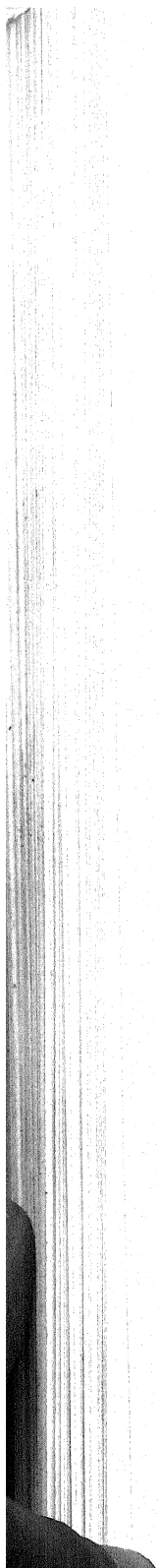
Tree No.	Location of leaves	Degrees of freedom	Sums of squares	Mean square	Log _e (mean square)	Difference	1/n	Sums
1.....	{Barren part.....	153	34,036.55	222.46	5.4048	} 0.0907	{0.006536	} 0.014473
	{Fruitful part.....	126	25,598.82	203.17	5.3141		{.007937	
4.....	{Barren part.....	112	35,639.40	318.21	5.7625	} .1048	{.008929	} .017858
	{Fruitful part.....	112	39,565.68	353.26	5.8673		{.008929	
5.....	Barren Tree.....	130	20,708.28	159.29	5.0701	} .1031	{.007692	} .016388
6.....	Fruitful tree.....	115	16,513.26	143.59	4.9670		{.008696	
5.....	1934 wood.....	99	37,681.37	380.62	5.9419	} .3557	{.010101	} .022446
6.....	do.....	81	21,602.37	266.70	5.5862		{.012345	

Consider only the last comparison shown in table 2, in which the greatest difference exists. The difference of the logarithms is 0.3557. Therefore, z is 0.1779, or half this difference. The variance of z amounts to one-half the sum of the two reciprocals, and is 0.01122. The standard deviation of z is then the square root of this half sum, which is 0.1059.

The greater mean square for tree 5 is significant, since z is 1.68 times greater than its standard deviation. The differences shown in the other three comparisons do not reach the level of significance. Larger numbers of observations would be required thoroughly to test and establish their reality. The data suggest, although but slightly, greater variability in the mutants with respect to leaf area.

SUMMARY

A preliminary study has been made of variation and correlation in leaf area in bud mutants of the Montmorency cherry. The results furnish a mere indication of greater variability in the mutant than in the normal form and they suggest the probability that the variance in the mutant, as determined by intraclass correlation, is to a higher degree concomitant. The results give no apparent support to the hypothesis that a bud mutant may vary more heterogeneously than the normal form and therefore be especially predisposed to give rise to other mutations.



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THE DEVELOPMENT OF THE FRUIT OF *MACADAMIA TERNIFOLIA*¹

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INTRODUCTION

During the course of various investigations in progress at the Hawaii Agricultural Experiment Station on growth and reproduction in *Macadamia ternifolia* F. Muell., close observations were made on the structure of the fruit. As a result of these observations, considerable doubt arose as to the accuracy of the generally accepted classification of the fruit as a drupe and of the shell of the nut as an endocarp or putamen. Von Mueller (6, v. 6, p. 191),³ who named the species, describes the fruit as possessing a horny pericarp with a rather swarthy exterior and a very smooth yellow and date-brown interior, the testa being thin and membranous. Engler (3) defines the fruit as a drupe with a fleshy external layer and a thicker, harder inner layer. Bailey (1) describes the fruit as having a two-valved leathery exocarp, the endocarp being smooth and shining, thick, and very hard. According to Bentham and Hooker (2, v. 3, p. 178), it is a subglobose, indehiscent drupe with a fleshy pericarp and a thick, hard endocarp.

The present paper presents the results of an investigation of the anatomy of the fruit and its parts. The study was nearing completion when an article by Francis (4) was brought to the writers' attention. Francis (4, p. 43) makes the statement: "A considerable amount of confusion exists in the descriptions of the fruit in systematic, botanical literature." He advances evidence in support of the fact that the nut is truly a seed and the fruit in which it is contained is not a drupe but a follicle. According to Francis, the shell of the nut is not endocarp but is the combined testa and tegmen. The present writers' investigations, on the other hand, show that the shell is made up of testa alone and that the inner integument does not develop into a seed coat. Whereas Francis based his studies mostly upon the mature fruit, the writers' observations covered the development of the fruit from the early flower-bud stages to maturity.

By reporting this work and reviewing the Australian reference, it is hoped that attention will be focused on the inaccuracies of current terminology and that the correct terms may be put into scientific use. While it is likely that in common usage and in the trade the macadamia will always be called a nut, it is important that the investigator have an accurate conception of the material that he is handling.

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² The writers take pleasure in acknowledging their indebtedness to Dr. A. J. Eames, of Cornell University, for his helpful discussion and valuable criticism.

³ Italic numbers in parentheses refer to Literature Cited, p. 406.

MATERIALS AND METHODS

The macadamia is represented in Hawaii by *Macadamia ternifolia* F. Muell. and its botanical variety *integrifolia* (Maiden and Betcher) Maiden (5, v. 1, p. 217). The differences between the species and its variety are chiefly in size of tree, degree of pubescence, color of flower, shape and size of leaf, and surface of shell. Because these characters have no bearing upon the fundamental structure of the fruit, the materials used in this study were taken from mature seedling trees of the variety *integrifolia*.

The material was killed and fixed in a formalin-alcohol-acetic-acid solution (3 ml. of 40-percent formalin, 90 ml. of 50-percent alcohol and 7 ml. of glacial acetic acid). The long, thick-walled hairs on the pistil and the sclerenchymatous tissues in the fruits necessitated treatment with 48-percent hydrofluoric acid over a period of 3 to 4 weeks for the former, and 5 months or longer for the latter. After a thorough washing in running tap water, the material was processed through Randolph's (7) *n*-butyl alcohol series and embedded in paraffin with a melting point of 56° to 58° C. Sections for the flower studies were cut at 10 to 12 μ ; for the fruit studies, at 10 to 15 μ .

A 0.3-percent solution of Delafield's haematoxylin in 50-percent alcohol proved to be the most satisfactory stain. The sections were stained for 30 minutes, followed by destaining in a dilute solution of hydrochloric acid for 5 to 10 minutes. Other staining combinations were generally unsatisfactory because of the retention of the stains in many of the cells of certain tissues.

Francis (4) performed a number of microchemical tests upon the contents of the heavily staining cells and concluded that the substance is probably a tannin or one of its derivatives, possibly phlobaphene. These contents in all cases give typical tannin reactions with both a ferric chloride and a 1-percent chromic-acid solution. In untreated sections, the cells appear to be filled with a dense, apparently colloidal substance varying from reddish brown to yellow. These cells are evident in the earliest stages of bud development and increase in number as the development of the tissues proceeds. Aside from Francis' suggestion that the substance may be phlobaphene, no attempt has been made to analyze it, nor does there seem to be any satisfactory method of removing it from the cells. As a result, the cells are rendered opaque under the microscope even when the sections have been cut very thin.

In addition to the microchemical tests upon the tannin deposits, Francis made two tests on the green pericarp tissue for the presence of a cyanogen. The first test with Guignard's sodium picrate paper gave a fairly positive reaction; the second with a 0.3-percent aqueous solution of mercurous nitrate gave a deposit of metallic mercury in the parenchyma cells, indicating the presence of a cyanogenetic glucoside or a labile compound.

The cell walls of the exocarp and testa of the fruit assume an intense reddish-violet color upon treatment of sections with phloroglucin and a red color when tested with Maule's reagent (8). These reactions indicate the presence of lignin. The lumina of cells in these tissues contain dark-colored deposits of a substance which gives a positive tannin reaction with ferric chloride.

OBSERVATIONS

DESCRIPTION OF THE FLOWER

The flowers of *Macadamia ternifolia* are small, about 12 mm. long at anthesis, perfect and apetalous (fig. 1, *B*), but having four petaloid sepals. They are borne in groups of three or four on pedicels about 3 mm. in length, along the rachis of a spikelike raceme. The pistil is superior, and the stamens are perigynous, being affixed with short filaments to the throat of the floral envelope. The pistil is surrounded at its base by an irregular-margined, glabrous disk (fig. 1, *C*), and it is composed of a single carpellate ovary that is densely pubescent and a style that is glabrous. The ovary contains two suspended, sessile, orthotropous ovules on the margins of its single ventral suture. Before the perianth exposes the anthers, it splits

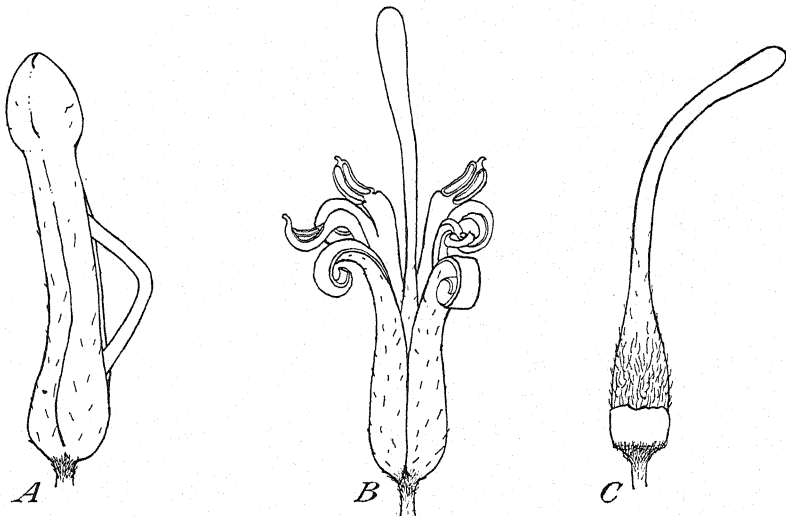


FIGURE 1.—*Macadamia* flower: *A*, The mature bud showing the perianth splitting to release the style; *B*, the flower in anthesis showing perianth, stamens, and style; *C*, the pistil with perianth removed to show the pubescent ovary, the pubescent style, and the glabrous disk.

along one side exposing the club-shaped style and its small stigma (fig. 1, *A*).

Transverse sections of the flower bud show the arrangement of the perianth parts to be valvate, the edges of the four sections joining one another by means of interlocking epidermal cells (pl. 1, *A*). The perianth envelope is regular and minutely pubescent. At anthesis its reflex points spread open to release the four two-celled anthers of the perigynous stamens.

A study of the vascular system of the flower shows that three traces, one dorsal and two ventral, branch from separate gaps in the stele of the receptacle into each of the four perianth parts (fig. 2, *C*, *D*). This three-trace condition indicates the sepal nature of the perianth. The single bundle of the stamen passes off directly after the dorsal bundle of the perianth part, to which it is adnate. The two bundles run parallel to the point at which the stamen is no

longer attached to the perianth. Vascular supply to the disk precedes that to the carpel and consists of several small strands containing very little xylem. The function of this disk is probably glandular. The carpel has five or seven main bundles (fig. 2, A, B). The exact number of traces derived from the stele is difficult to determine because the bundles all arise at about the same level and branch readily after their departure. The extra traces lie between the dorsal and ventrals on either side. The vascular system of the ovules is derived from the ventral bundles (fig. 2, B).

INITIAL STAGES IN FRUIT DEVELOPMENT

Ovule formation is evident in buds about 1.0 to 1.5 mm. in length (pl. 1, A). A well-defined suture can easily be traced throughout the entire length of the short pistil. On either margin of the suture a mass of meristematic tissue protrudes into the ovarian cavity.

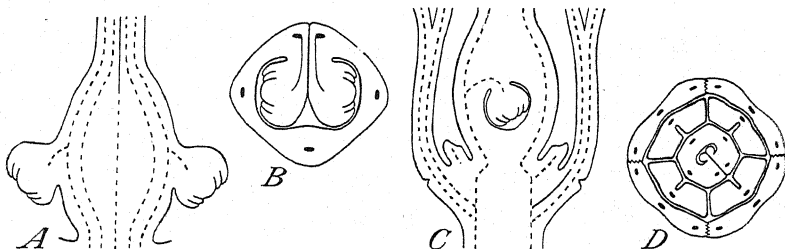
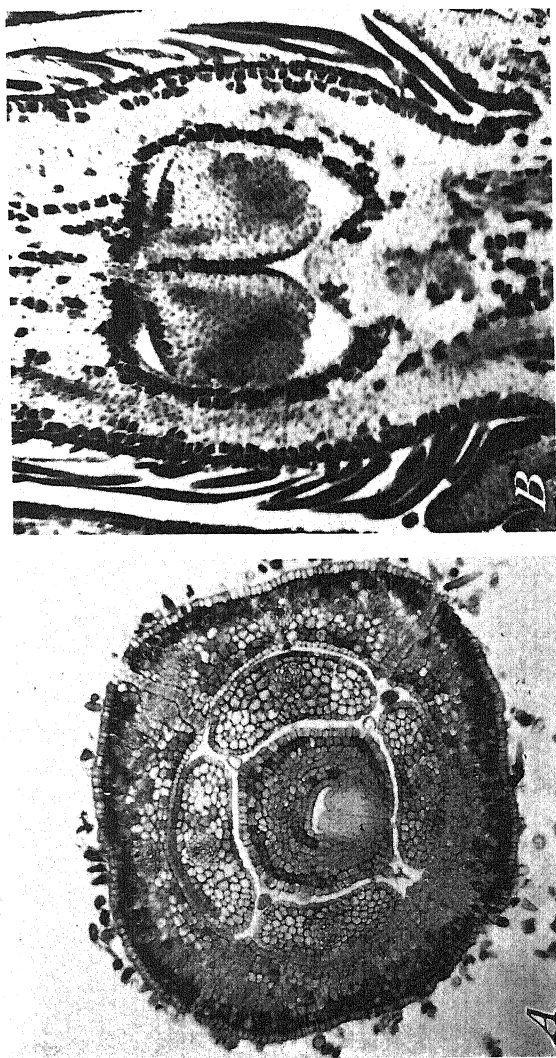


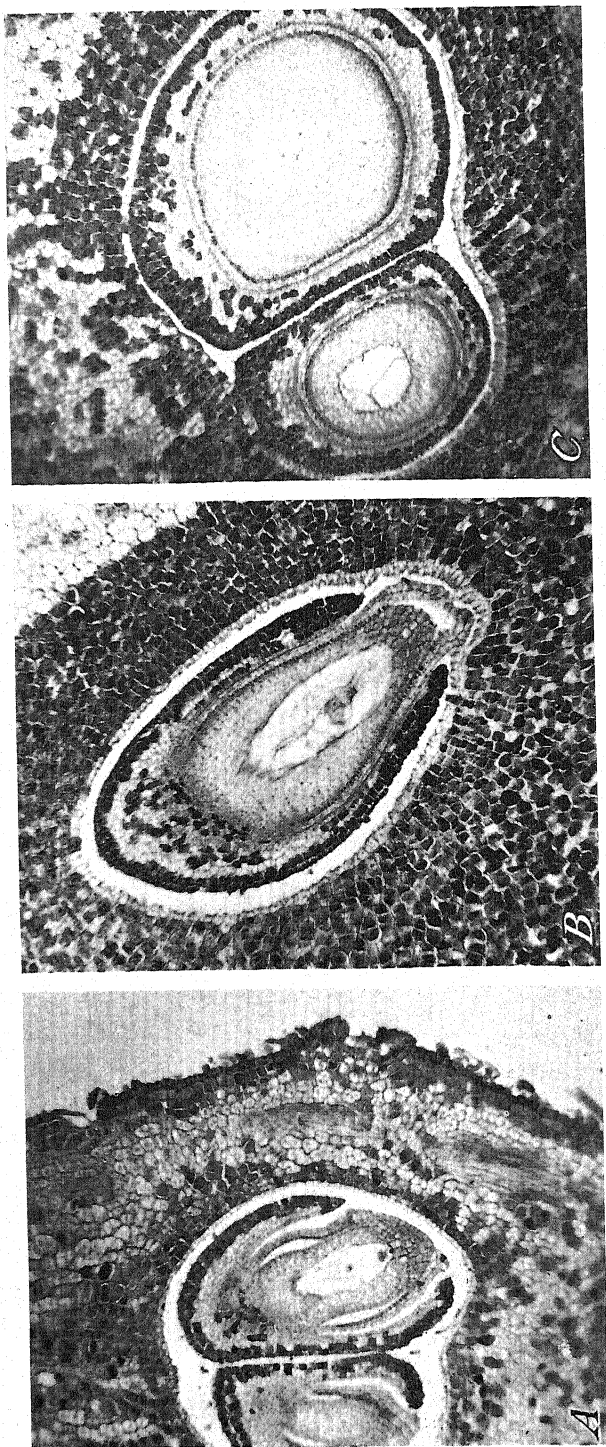
FIGURE 2.—Vascular system of *Macadamia ternifolia* flower: A, Carpel split ventrally and spread open, showing trace number and venation; B, transverse section; C, median longitudinal section of base of flower showing course of traces to floral organs—dorsal and only one ventral trace shown in carpel; D, transverse section.

These masses of ovule tissue increase in size by rapid cell division until an average size of about 86 by 122μ is reached, when the inner and outer integuments appear as two small folds of tissue protruding from the base of the developing ovule (pl. 1, B). All of the cells of the ovule are typically meristematic at this stage, having diameters essentially alike, thin cellulose walls, large nuclei, and small vacuoles. The outer integument is shorter and thicker than the inner which, at this stage, is two or three layers thick, tapering off to a single layer as it approaches the micropylar end. The ovules are crowded against the ovary wall at the top and sides so that both integuments remain closely associated with the nucellus up to the time that megasporogenesis begins.

By the time the flower bud has fully developed, megasporogenesis has been completed, followed by the elongation of the nucellus and the development of an embryo sac. The integuments have also elongated and now invest almost the entire nucellus (pl. 2, A). Deposits of tannin, or tannin derivatives, become abundant in the two outermost layers of the outer integument; the nuclei of the cells in these layers persist for a while after they have become filled but later disintegrate, leaving only spaces or lumina.



Macadamia ternifolia: A, Transverse section through a bud 1.5 mm. long showing the mass of meristem protruding into the ovarian cavity initiating ovule formation. Note interlocking of epidermal cells between perianth parts. B, Dorsal view of longitudinal section through the ovary of a bud 4 mm. long showing the two pendulous ovules with the outer and inner integuments being initiated. A, B, $\times 100$.



Macadamia ternifolia: A, Longitudinal section through ovary just prior to fertilization, showing the position of the integuments. B, Longitudinal section of portion of young fruit 2 mm. in diameter showing increase in size of the testa and the inner integument with its opaque inner epidermis. Dark-colored layer of pericarp is endocarp, light-colored portion in upper left corner is exocarp; the light central area is nucellus tissue surrounding the embryo sac in which endospermic nuclei are present. C, Transverse section showing a normal and an aborting seed. Note prominence of inner epidermis of ovary wall. A-C $\times 100$.

INTERMEDIATE STAGES IN FRUIT DEVELOPMENT

After fertilization, the perianth and disk of the flower drop off, leaving only the pistil on the pedicel. As the ovary develops, the long style gradually dries back and falls off, only the remnant of the stylar projection remaining on the fully developed fruit.

A longitudinal section of the ovary at approximately the time of fertilization shows that the carpel wall is made up of a thick layer of parenchymatous and vascular tissues with an outer epidermis from which arise many thick-walled hairs and an inner epidermis of small active cells which lines the ovarian cavity (pl. 2, *A*). This inner epidermis is conspicuous in the developing fruit (pl. 2, *B*, *C*) but disintegrates before maturity is reached. The suture is prominent as a small furrow extending along one side from the apex to within a few millimeters of the base. As seed development goes on within, the ovary wall increases in thickness and differentiates into two layers, the exocarp and the endocarp (pl. 2, *B*). The endocarp is composed of parenchyma cells only, but the immature exocarp is composed of both parenchyma cells and primary vascular elements.

The outer and inner integuments expand slightly to allow for development of the embryo tissues. The inner integument, three cell layers thick, increases in length to encompass the micropylar portion of the nucellus and protrudes into the micropyle (pl. 2, *B*, and Fig. 3, *B*). Further differentiation in this tissue is limited to the inner epidermal layer which becomes opaque from impregnation with what appears to be tannin substances. The tissue remains intact through the early stages of maturity and in later stages it is present as a layer of disintegrated tissue adhering to the white portion of the inner surface of the shell or testa (fig. 3, *E*). The mature embryos tend more or less to cling to the white enamel portions of shell or outer seed coat. This adherence may be due in part to the presence of the vestigial inner seed coat.

The outer integument, which should now be referred to as the seed coat or testa, does not appear to be active at this stage; but when the embryo is differentiating to form cotyledons and the plumule-radicle axis the cells of the testa multiply and enlarge, and the tissue completely surrounds the embryo. Development is particularly active in the chalazal region and accounts for the thicker layer of shell at this point. The meristematic cells forming the inner epidermis of the testa divide to form a tissue three to five cells thick. These cells form the white enamel portion of the inner layer of the shell (fig. 3, *D*, *E*).

Endosperm development is rapid during a period of from 4 to 8 weeks after fertilization. In its early stages the endosperm is multinucleate, the nuclei and cytoplasm being limited to a peripheral layer around a central vacuole. By the end of approximately 8 weeks, cell walls have formed and the gelatinous endosperm occupies all but a very small portion of the embryo sac. The nucellus is not completely absorbed, several layers of it persisting in the developing seed up to the last stages prior to maturity (fig. 3, *D*). Although at first the development of the embryo is more gradual than that of the endosperm, by the end of approximately 20 weeks the cotyledons completely replace the endosperm-tissue.

The two ovules appear to be alike in every respect until the time of fertilization, but usually only one of them develops in the fruit (fig. 4). The causes of the abortion of one are still unknown, although abortion appears to be a varietal characteristic. The aborted structure may be found adhering to the endocarp or, in various stages of development, clinging to the normal seed.

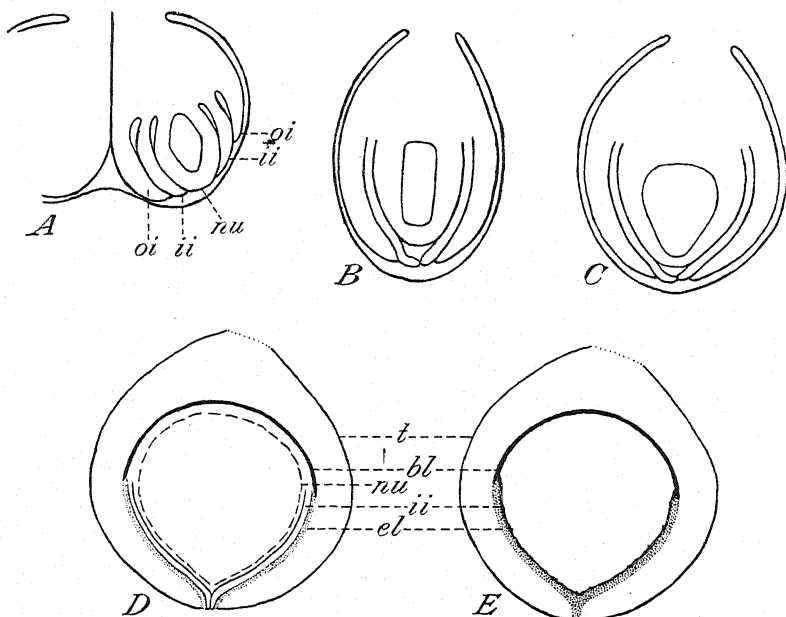


FIGURE 3.—*Macadamia ternifolia*: Longitudinal diagrams showing development of outer integument and changes in inner integument and nucellus tissue from bud stage to mature seed: A, Bud stage showing positions of two ovules, outer integument, inner integument, and nucellus tissue; B, fertilized ovule showing inner integument protruding into the micropyle; C, intermediate stage with nucellus being absorbed by developing embryo; D, shell of seed before it hardens showing remnants of nucellus, the white enamel layer developing from inner epidermis of testa and the brown layer; E, hardened shell with disintegrated inner integument clinging to white enamel portion. *oi*, Outer integument; *ii*, inner integument; *nu*, nucellus; *t*, testa; *bl*, brown layer; *el*, enamel layer. A, B, $\times 100$; C $\times 90$; D, E, $\times 22$.

THE MATURE FRUIT

The entire mature fruit is a follicle, globose, and slightly oblique in shape, which, when ripe, dehisces along the ventral suture (fig. 4, C). The seeds are globular (fig. 4, D) except when both ovules have developed, in which case they are hemispherical, with one located in each valve of the dehiscent pericarp.

The kernel of the seed is composed of two semiglobose cotyledons enclosing a small, subglobose plumule-radicle axis.

THE PERICARP

The dehiscent pericarp consists of two distinct layers of tissue, a fibrous exocarp with a dark-green, fairly smooth exterior and a softer, thinner endocarp. The exocarp has an epidermis overlying a thin layer of chlorophyll-bearing cells, the main body being made up

of parenchyma tissue in which are embedded numerous freely branching vascular bundles. The endocarp is devoid of bundles, and its parenchyma cells are filled with dark tannin-like substances (pl. 2, *B*).

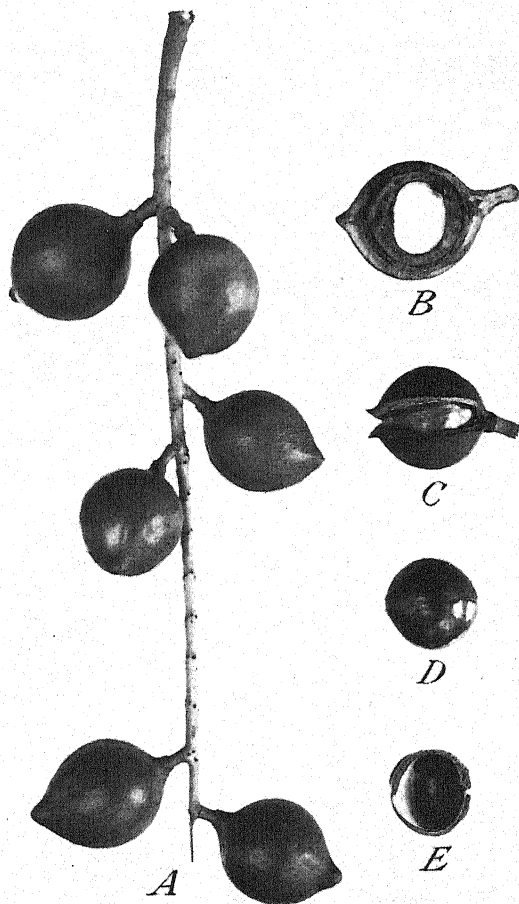


FIGURE 4.—*Macadamia ternifolia*: *A*, Mature green fruits on rachis; *B*, mature green fruit split in half showing the embryo and its enclosing shell within the pericarp; *C*, split pericarp with mature seed; *D*, seed; *E*, empty half shell showing the cream-colored portion of the inner surface (micropylar end at left) and dark-brown portion (chalazal end at right). $\times \frac{1}{2}$.

THE SEED COAT

The so-called nut contained in the pericarp is a true seed having a seed coat, a hilum, and a micropyle (fig. 4, *D*, *E*). The seed coat has developed from the outer integument into the hard, bony shell, generally mistaken for an endocarp.

The testa, as mentioned above, is made up of two distinct layers. The outer, thicker portion is a very hard sclerenchyma tissue of fiber and stone cells. The elongated fiber cells predominate and occur in strands or sheets extending both longitudinally and radially. The stone cells vary considerably in size and shape and contribute to the rigidity of the tissue by forking and interlocking (pl. 3, *B*). The walls of both types of cells are highly lignified and abundantly pitted. Fine vascular bundles with spirally thickened elements are also seen scattered throughout the tissue.

The inner layer of the shell is about one-fifteenth as thick as the outer layer. About one-half of the shell interior, toward the hilar

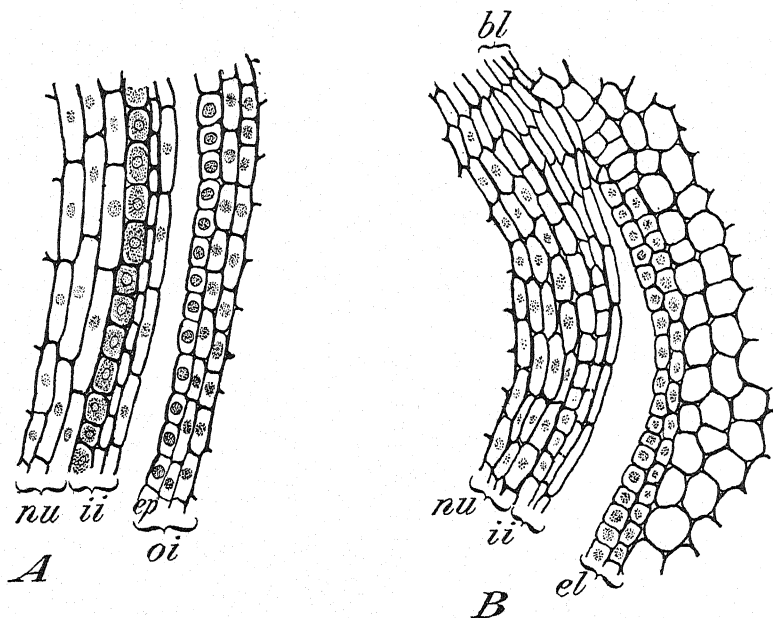
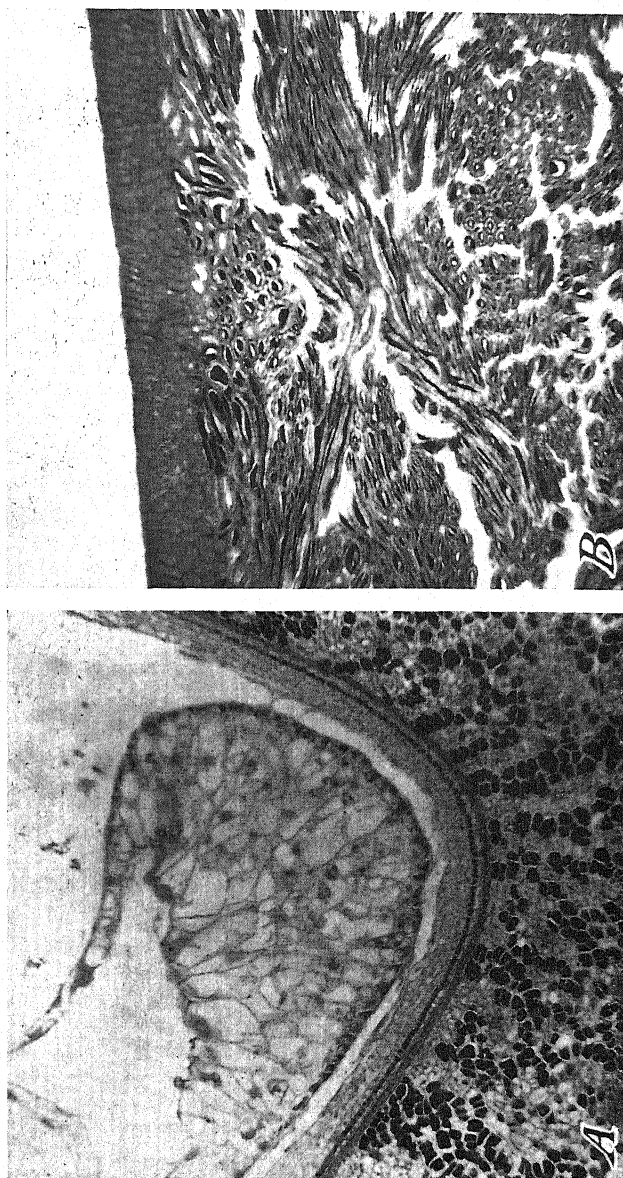
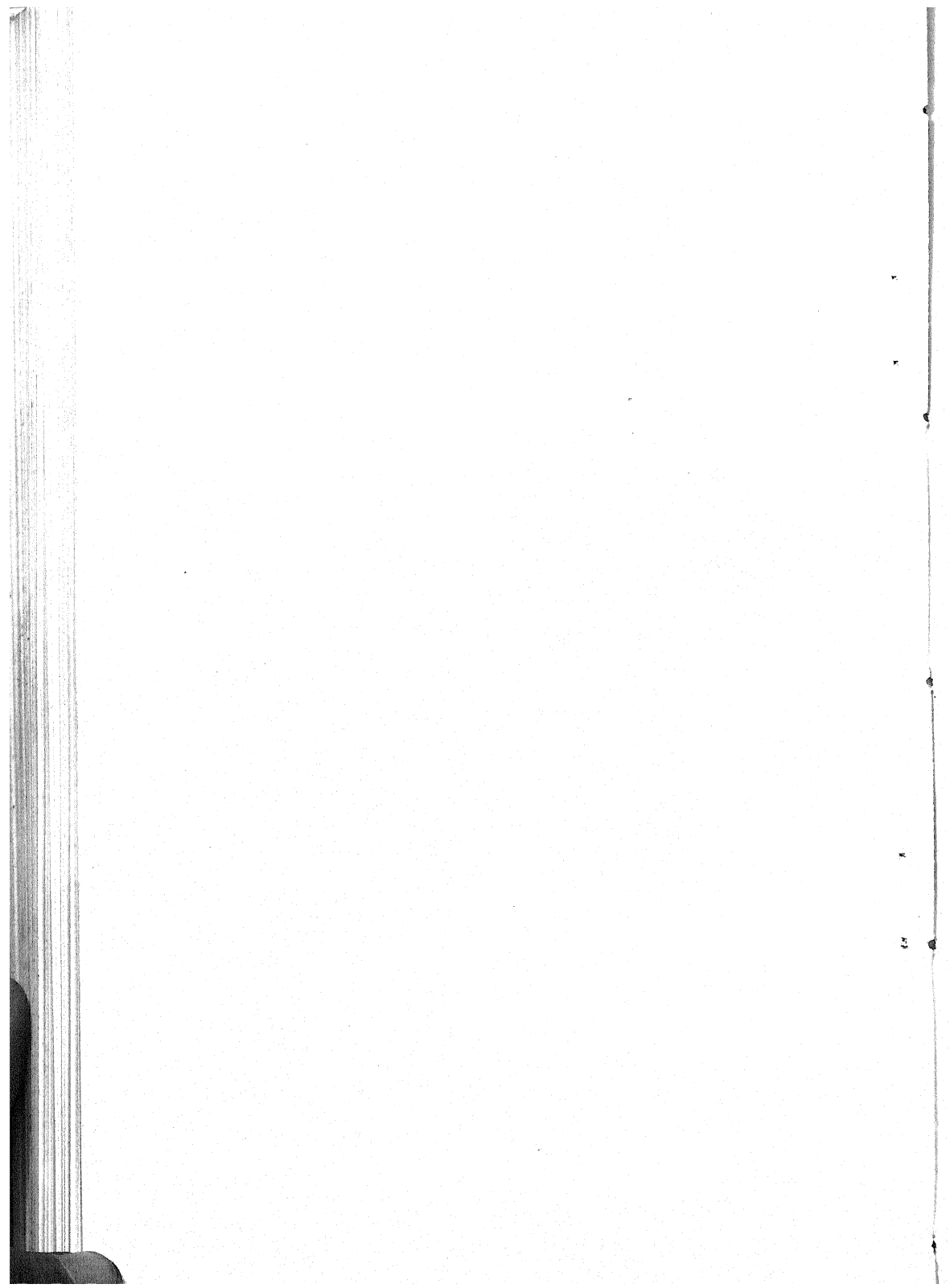


FIGURE 5.—*Macadamia ternifolia*: *A*, Diagram of section of young seed showing cells of nucellus, inner integument, and inner layers of outer integument. The heavily shaded layer is filled with tanninlike deposits. *B*, Section from older seed through region where the integuments come together showing development of enamel layer from epidermal cells in outer integument, and cells of brown layer. *nu*, Nucellus; *ep*, epidermis; *ii*, inner integument; *oi*, outer integument; *el*, enamel layer; *bl*, brown layer. $\times 500$.

end, is shiny and dark brown while the half toward the micropylar end is enamellike and cream-colored (fig. 4, *E*). Francis (4) believed this entire inner layer to be tegmen developed from the inner integument, but the sections made by the writers show that it is a part of the testa. Differentiation of the inner layer begins at that point in the chalazal region where the outer and inner integuments originate (fig. 5, *B*). The brown section, which develops around the embryo at its apical or broader portion, is composed of somewhat flattened and compacted cells, filled with a brown deposit similar to that which fills the cells in the outer layer of the testa. The cream-colored section covering the lower or basal portion of the embryo is composed of cells derived from the inner epidermis of the outer integument (fig. 5, *A*).



Macadamia ternifolia: A, Longitudinal section of young seed showing testa, nucellus, and endosperm tissue. B, Section of a mature shell showing the arrangement of the stone cells of the testa and the dense polygonal cells of the white enamel layer. Rupture of tissue is caused by cutting. $\times 100$.



These cells occupy a position parallel to that of the undeveloped inner integument which lies within it (fig. 3, *E*). Francis made a very complete and detailed study of the compact polygonal cells making up the enamel portion. The walls seem to be unstratified and without abundant pits. The granular material contained in the cells is lignocellulose. In prepared sections of this tissue, each cell has a small, angular, clear space, which, according to Francis (4, p. 47), "indicates a position in the cell occupied by a crystal of calcium oxalate, which appears to have been dissolved either by the treatment involved in the preparation and mounting or by the natural processes of the maturing seed."

DISCUSSION

A drupe falls under the general classification of simple fleshy fruits. It is derived from a single carpel and is usually one-seeded. There may be three distinct layers in the ripened pericarp, in which case there is a thin exocarp forming the skin, a fleshy mesocarp, and a stony endocarp; or, as is more common, there may be only two layers, a fleshy exocarp and a stony endocarp. A nut is usually defined as a hard, dry, indehiscent, one-seeded and one-celled bony fruit even if it represents a compound ovary. A follicle on the other hand is a dehiscent, unicarpellate fruit which splits along one suture at maturity and contains one or more seeds. By definition, therefore, the macadamia fruit is not a drupe but is a follicle, and the "nut" is not truly a nut but is the seed contained in the follicle.

While it hardly seems likely that in common usage and in the trade the macadamia "nut" will ever be called anything else, it should be borne in mind that it is properly a seed and that the shell is not putamen but is a well-developed testa. It may be seen how the unusually hard, thick shell of the seed could be mistaken for the stone of a drupe by the early systematic botanists and how the name of nut could be applied to it in common usage.

The development of the outer integument into a thick, hard testa is not peculiar to the macadamia alone, for the hard shell of the Brazil nut (*Bertholletia excelsa*) also is a seed coat and not a pericarp tissue.

The brown smooth portion and white enamel layer of the shell were interpreted by Francis as being a tegmen developed from the inner integument. The observations of the writers lead them to believe that these portions are actually a part of the testa (outer integument). The enamel layer is developed from the cells of the inner epidermis of the outer integument. The cells of the brown layer develop from the seed-coat tissue at the base of the ovule and form a line which separates the developing kernel from the testa.

SUMMARY

Anatomical investigations indicate that the fruit of *Macadamia ternifolia* F. Muell. is a follicle and not a drupe. The pericarp is dehiscent along a single suture and is made up of a thick, leathery exocarp and a thin, soft endocarp. The so-called nut is a true seed having one seed coat that develops from the outer integument of the ovule, a hilum, and a micropyle.

The shell of the nut is the testa. The brown and white layers lining the inside of the shell are developed from the testa.

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SOIL CHARACTERISTICS, TOPOGRAPHY, AND LESSER VEGETATION IN RELATION TO SITE QUALITY OF SECOND-GROWTH OAK STANDS IN CONNECTICUT¹

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INTRODUCTION

Approximately 56 percent of the area of Connecticut is in forest and brushland. While the species growing within its borders are many, the predominant forest type is oak. Because of the importance of oak in Connecticut forests, it was deemed advisable to ascertain the relationship, if any, existing between growth of oaks (expressed as site quality or site index, and measured in terms of the height growth of dominant trees) and various topographic, soil, and vegetative factors.³ This study is primarily an extension of investigations begun with red pine and published in 1931 (10).⁴

As a recent publication by Aaltonen (1) includes an excellent review of the literature, it is unnecessary to discuss it in detail here. Taken as a whole, the findings are conspicuously dissimilar. Some investigators have observed some correlation between site quality and nutrient content of the soil; others have found none. In some cases the biological properties of the soil were found to be of marked significance. Practically all agree on the importance of soil moisture, and in many cases site quality varies with the proportion of silt and clay in the soil. In the case of sandy soils in the vicinity of Eberswalde, Germany, the presence of an underlying marl or clay layer within reaching distance of the tree roots frequently is the chief factor in controlling stand composition and yield, according to Ganssen and his coworkers, and Wiedermann, as reported by Aaltonen (1). In his English summary Aaltonen states:

Determination of the productivity of soil on the basis of its properties is one of the most important objects of forest soil research. At the same time, it is one of its most difficult problems, for it seems that the productivity seldom depends upon a single factor or even a few factors, but is usually a result of the combined actions of several factors.

Burns (3) was thinking along the same line when he wrote, "The amount of increment and the width of the annual ring in a tree's growth show its response to the physiological summation of all the factors of the site." Turner (19, 20), working in Arkansas, states that "soil features influencing available water seem to be more influential than any others in determining the rate of growth of pine trees" (19, p. 11). He found no correlation between occurrence of growth

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² Acknowledgment is made to Henry W. Hicock, assistant forester, Connecticut Agricultural Experiment Station, for advice and assistance, especially in the calculation of the site indices.

³ The field work was done in 1930, principally by Henry Bull, formerly assistant in forestry, Connecticut Agricultural Experiment Station.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 427.

and any single soil factor; but he did find a correlation with certain "site-soil complexes."

One may sift from the many papers on this subject at least two fairly well established conclusions that are generally applicable in the principal forest regions of Europe and the United States: (1) Available moisture is usually the factor of prime importance in all but heavy or poorly drained soils; and (2) lime is usually second in importance. Climate and soil vary so greatly that each forest district and in some cases each individual stand presents its own peculiar problems which require separate investigation and solution.

With respect to the oak stands in Connecticut, Frothingham in 1910 (6) made a study of growth, uses of lumber, and methods of management of second-growth chestnut, oak, and mixed hardwoods. He did not study the soils but merely stated that "soil conditions are largely responsible not only for differences in type but also for variations in the rate of growth within the same type." At that time chestnut occupied the better soils, and the oaks, especially black, scarlet, and chestnut oak, were confined largely to dry upper slopes and ridge tops. Since the disappearance of the chestnut its place has been taken principally by red oak. As Frothingham's yield data have been largely superseded by the recent work of Schnur (16), no further reference to the former will be made here.

In 1926 and 1927 Hicock and others (10) attempted to ascertain the relationship between soil type and stand composition in four State forest tracts. The results were largely negative, principally for the following reasons: Probable biological equivalence of two or more soil types; generally favorable climate; and effect of fire and other factors brought about mainly through the intervention of man.

METHODS

SELECTION OF PLOTS

The 76 stations (hereinafter called plots although they were of no definite size or shape and were not marked off) used in this study were taken wherever there was an even-aged stand in which the great majority (80 percent or more) of the dominant trees were oaks. Stands were considered even-aged if the dominants did not vary by more than 10 years and were reasonably uniform in height. On at least 80 percent of the plots the range of ages of dominants was not more than 4 to 5 years. Five species of oaks are well represented, namely—white (*Quercus alba* L.), red (*Q. borealis maxima* (Marshall) Ashe), black (*Q. velutina* La M.), scarlet (*Q. coccinea* Muench.), and chestnut (*Q. montana* Willd.). Two other species—swamp white (*Q. bicolor* Willd.) and pin oak (*Q. palustris* Muench.)—were present to a limited extent and some data on these are included. The number of trees measured on each plot averaged about 20, although it varied from less than 10 to about 50.

FIELD MEASUREMENTS AND OBSERVATIONS

Measurements in the field included height, diameter at breast height (d. b. h.), and age of dominant and codominant oaks, separately for each species. Intermediate and ground-cover vegetation were listed and estimated as to density and uniformity of distribution. Careful notes were taken on the topography and related environmental fea-

tures, character of the forest floor,⁵ and soil description. In addition, the mineral soil to a depth of about 5 inches was sampled for study in the laboratory.

CALCULATION OF SITE INDICES

The height curves and formulae of Schnur (16) (obtained through the Northeastern Forest Experiment Station prior to publication) were used as a basis for the calculation of the site indices. In determining total age, 3 years was added to the age at breast height. Schnur, on the other hand, used a correction factor of only 2 years, although this was not known by the writer until the appearance of Schnur's bulletin. The data have not been revised to correspond with Schnur's in this respect, for the following reasons: (1) Many trees in this study came from seedlings that grow slowly, while apparently most of those represented in the Forest Service study were sprouts; (2) on the whole, growing conditions in Connecticut are not quite so favorable as those in most of the areas covered by the Forest Service study; and (3) the differences in site index resulting from the 1-year difference in estimated age are quite small (average difference in the first 10 plots recorded in this study was 0.8 foot).

The figures for the height of the average tree (average basal area) used in the calculations were obtained by plotting the measured heights against the measured diameter at breast height, and then using the diameter at breast height by basal area to determine the height.

The principal equation used in calculating site index was:

$$I \text{ (site index)} = 62.7 + 8.37 \frac{H - Ha}{\sigma a}$$

where

H = average dominant height of the stand in question

Ha = average height at any age a

σa = standard deviation of height about the average at any age a

A plotting of these 76 plots on the Forest Service set of curves indicates that the trend of an average curve representing all of these plots does not follow very closely the trends of the Forest Service curves. Since the plots were taken where suitable stands could be found and do not necessarily constitute a good random sample, this lack of agreement is not significant. However, since the Forest Service found no significant differences between oaks from a region reaching from Missouri to Maryland and from Tennessee to Michigan, it is reasonable to believe that Connecticut oaks would not be an exception in this respect. Therefore, for present purposes we can assume applicability to Connecticut conditions.

SOIL STUDIES

The following laboratory analyses were made on the soil samples collected in this study: Total nitrogen, loss on ignition, reaction (pH), exchangeable hydrogen,⁶ total base capacity,⁸ percent saturation,⁶ conductivity,⁷ moisture equivalent, total colloids,⁸ and soluble nutrients by quick test.⁹

⁵ Forest floor = the whole organic debris, including litter, F and H layers.

⁶ Determined by the $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2 - \text{NH}_4\text{Cl}$ method of Pierre and Scarseth (15).

⁷ Electrical conductivity of a water suspension.

⁸ Determined by the hydrometer method of Bouyoucos (2).

⁹ Morgan's Universal soil test.

DESCRIPTION OF THE REGION

Connecticut lies at the northeastern edge of the upland oak region, with some intermingling of the whitepine types in the northern part of the State. The forest associations generally recognized (14) are: Red cedar-gray birch association, 20 percent (of the total forested area); hardwood association, 70 percent; hemlock-hardwood association, 5 percent; and swamp association, 5 percent. In the hardwood association the oaks predominate.

Climatically, Connecticut is characterized by relatively long, cold winters and rather short, humid summers. The mean annual temperature is 47° to 49° F.; mean maximum in winter, 31° to 38°; mean minimum in winter, 15° to 20°; length of growing season, 150 to 170 days; mean annual precipitation, 44 to 46 inches, rather evenly distributed throughout the year. In table 1 are given the temperature and rainfall data by months.

TABLE 1.—*Temperature and rainfall data for Connecticut¹ by months and the annual average or total*

Month	Temperature			Mean precipitation	Month	Temperature			Mean precipitation
	Mean	Mean maximum	Mean minimum			Mean	Mean maximum	Mean minimum	
	° F.	° F.	° F.	Inches		° F.	° F.	° F.	Inches
January	26.6	35.4	18.1	3.84	August	68.6	78.7	58.3	4.27
February	26.1	35.2	17.0	3.77	September	62.3	72.9	52.0	3.75
March	35.0	44.7	26.2	4.11	October	51.6	62.6	41.5	3.63
April	45.9	56.5	35.6	3.69	November	40.3	49.5	31.3	3.62
May	57.1	68.3	45.9	3.66	December	29.6	38.0	21.3	3.84
June	65.6	76.4	54.6	3.31					
July	70.6	81.0	60.4	4.17	Average or total	48.3	58.3	38.5	45.65

¹ Average means from 7 stations well distributed over the State as follows: Colchester, Cream Hill (Cornwall), Storrs, New Haven, Southington, Voluntown, Waterbury. All records for periods of 30 years or more.

Since we are attempting to apply to Connecticut site-index curves obtained by the Forest Service in the region extending from Maryland to Missouri and from Michigan to Tennessee, it is well to compare climatic conditions. This is done in table 2 through the use of Jenny's data (12). (A low rain factor and a high *NS* quotient indicate favorable growing conditions.) Here we see that in comparison with the other areas, Connecticut has a slightly higher rainfall but a lower mean temperature, resulting in a decidedly higher rain factor. Relative humidity is practically identical, but the *NS* quotient is higher. The net result is a somewhat less favorable growing condition than the average of the other areas.

Elevations in Connecticut range from sea level to a maximum of 2,355 feet, with the greater portion of the land lying between 100 and 1,500 feet.

The soils of Connecticut belong to the Brown Podzolic group which is found only in the Northeastern States south of the Podzol area of New England.

Essentially, the Brown Podzolic soil is an imperfectly developed Podzol having, in timbered areas, an organic mat on the surface and a very thin gray leached horizon just below it—usually less than an inch thick. The B horizon is largely

yellowish brown in color and has only the beginnings of a dark-brown orterde just below the gray A horizon. The total depth of the solum is usually less than 30 inches although it exceeds that depth in places (21).

Fine sandy loams predominate in the forested areas and are prevailing acidic, being derived principally from granitic and dioritic gneisses and schists and, in the central lowlands, from red sandstones and shales. Soils from basaltic traprock constitute a very small proportion of the whole.

TABLE 2.—*Comparison of climatic factors in Connecticut and certain other States*
[Data taken from Jenny (12)]

Places compared	Precipitation		Temperature		Relative humidity	Rain factor Precipitation, mm. Temperature, ° C.	N.-S. quotient ¹
	Millimeters	Inches	° C.	° F.			
Connecticut:							
Hartford	1,175	46.26	9.78	49.6	71.5	120	457
New Haven	1,163	45.78	9.94	49.9	73.0	117	472
Average	1,169	46.02	9.86	49.75	72.25	118.5	464.5
Average of 33 stations in 10 States: ²	1,002	39.44	11.84	53.31	72.50	84.6	357

¹ N.-S. (N.-S. = Niederschlagsmenge-Sättigungsdefizit) quotient = $\frac{\text{Precipitation}}{\text{Absolute saturation deficiency of the air.}}$

² Illinois, Indiana, Kentucky, Maryland, Michigan (south), Missouri, Ohio, Pennsylvania, Tennessee, and West Virginia.

Forest humus types vary from excellent mulls through granular mors¹⁰ to extreme fibrous mors (9). Greasy mor is not common and, in general, is found only under hemlock. By far the prevailing type under oak and mixed hardwood stands is a fairly good form of granular mor.

As is true elsewhere, the best areas are used for agriculture so that most of the land in forest or brush falls into one or more of the following categories: Rocky, shallow, steep, low fertility, excessively sandy or gravelly, swampy.

The history of land utilization is approximately as follows (5): Until about 1700 practically all of southern New England was forested with a large variety of hardwood species, some hemlock, and probably a little white pine in the northern portion. Then with the marked increase in settlement by the white man, much of the timber was cut and the land used for farming—including a great deal of rough and stony land that should never have been cleared. The maximum amount of land used for agriculture reached its peak between 1830 and 1850, after which, with the opening of the better farm land of the West, there began a gradual diminution of farming and slow reversion of the land to brush and forest.¹¹ This process has continued to the present day.

¹⁰ By "mor" is meant a type of forest humus in which the organic matter is practically unmixed with the mineral soil and is usually more or less matted or compacted.

¹¹ The normal succession (14) on the poorer sites is: Shrubs (such as *Myrica* and *Rhus*) → red cedar-gray birch → inferior hardwoods (dogwood, hophornbeam, blue beech, red maple, shadbush, chokecherry, sassafras, butternut, pignut hickory, bitternut hickory, and large-toothed aspen) → better hardwoods (red oak, scarlet oak, white oak, chestnut oak, black oak, white ash, shagbark hickory, mockernut hickory, black birch, paper birch, yellow birch, beech, yellow poplar, black cherry, white elm, sugar maple, basswood) → hemlock-hardwoods (climax). On the better sites the shrub stage is omitted.

The characteristic practice among forest and wood-lot owners is to clear-cut as soon as the trees are big enough to make cordwood and then allow the land to grow up again to forest in its own way without control or direction.¹² Such practice means that Connecticut is producing not more than a third of the wood that could be produced under intensive, intelligent silviculture (7).

PRESENTATION OF RESULTS

SITE INDICES

The data pertaining to age, height, and diameter at breast height, and site index are summarized in table 3. In table 4 is found the plot distribution by site index and age. Seventy-four percent of the plots have a site index between 50 and 70, and 78 percent lie between the ages 20 and 60. In the case of the Forest Service study, the corresponding values were 77 and 72 percent, respectively.

TABLE 3.—Averages and ranges of age, height, diameter-breast-high, and site-index data for forest plots studied

Item	Average	Range	Item	Average	Range
Age.....years	43.2	12-105	D. b. h. by basal area, inches.....	7.75	1.9-14.7
Height.....feet	53.2	17-81	Average height from curve.....feet.....	52.9	16-81
D. b. h.....inches	7.98	2.2-15.6	Site index.....	59.7	30-83

TABLE 4.—Plot distribution by site index and age

Total age (years)	Plots in site-index class—						Total	
	20-39	40-49	50-59	60-69	70-79	80-89		
	Number	Number	Number	Number	Number	Number	Number	Percent
10-19.....		1					1	1.3
20-29.....			4	4	3	1	12	15.8
30-39.....			5	6	3	1	15	19.7
40-49.....		2	8	9	1		20	26.3
50-59.....	1	4	6	1			12	15.8
60-69.....		1	5	2			8	10.5
70-79.....		1	1	2			4	5.4
80-89.....	1		1	1			3	3.9
90-99.....								
100-109.....			1				1	1.3
Total.....	2	9	31	25	7	2	76	
Proportion.....	Percent 2.7	Percent 11.8	Percent 40.8	Percent 32.8	Percent 9.2	Percent 2.7	Percent	100.0

When the site indices were segregated by species, the data summarized in table 5 were obtained. The black, red, and scarlet oaks ran somewhat higher in site index than the white and chestnut oaks. This is a little unusual, for white oak generally shows a more rapid growth than either black or scarlet oak. Red oak led in the number of plots. Of the several species investigated, only black oak was found in the site index class above 80.

¹² "On land which has been cut over, burned, grazed, or otherwise severely disturbed, but not to the exclusion of all former forest growth, hardwoods usually control the situation from the outset. As a rule inferior hardwoods predominate at first, later on giving way to hardwoods of the better type. On poor sites, however, the red cedar-gray birch stage may precede the hardwood stage * * *. In general, succession on this class of land is more rapid than on old field land" (14).

TABLE 5.—*Summary of site index by species*

[Number of plots in parentheses]

Site-index class	Black oak	Red oak	Scarlet oak	White oak	Chestnut oak	Swamp oak	Pin oak
30-39.....	-----	34.6 (1)	37.3 (1)	-----	32.7 (1)	-----	-----
40-49.....	48.9 (2)	45.0 (1)	49.1 (1)	46.1 (5)	48.0 (3)	-----	-----
50-59.....	57.9 (6)	55.1 (9)	54.9 (8)	56.0 (14)	52.5 (4)	59.0 (1)	-----
60-69.....	65.0 (9)	64.9 (16)	64.1 (14)	62.6 (11)	64.2 (4)	-----	61.4 (1)
70-79.....	71.9 (2)	75.4 (7)	74.8 (4)	-----	70.1 (1)	-----	-----
80-89.....	84.1 (2)	-----	-----	-----	-----	-----	-----
Average (of individual plots).....	63.9 (21)	63.0 (34)	61.5 (28)	56.8 (30)	54.9 (13)	-----	-----

CORRELATION OF SITE INDEX WITH CHARACTERISTICS OF THE UPPER MINERAL SOIL

When we attempt to correlate the site index of all plots with certain qualities of the soil as sampled (upper 5 inches of mineral soil), the results obtained are very conflicting, as the summary in table 6 shows. With total colloids (particles <0.05 mm.) and moisture equivalent, the correlation is negative; with total sands and nitrogen, slightly positive; and with pH value, very slightly positive. In no other case is there any correlation except in the quick tests where Ca, P, K, and $\text{NH}_3\text{--N}$ shows a positive correlation. However, in these tests, the data are characterized by extreme deviations within each site class which greatly reduces the significance of the means. Quick tests are only roughly quantitative and cannot be expected to reveal fine differences. (It should be mentioned that the quick-test values herein reported cannot be compared with similar data obtained at a later date for the reason that both the procedure and the color scale were changed. The data as given have value only for comparison.)

 TABLE 6.—*Summary of relation between site index and soil characteristics in all 76 plots*

Soil characteristic or constituent	Data for site-index class—						Correlation indicated
	20-39	40-49	50-59	60-69	70-79	80-89	
Average site index.....	33.2	46.4	56.0	64.3	74.3	81.9	
Plots..... number	2	9	29	27	7	2	
Total colloids..... percent	20.0	17.2	14.4	14.6	12.5	7.5	Inverse.
Total sands..... do	44.2	57.4	61.7	58.8	63.3	81.6	Positive.
Moisture equivalent..... do	26.3	23.4	20.5	21.3	19.5	10.3	Inverse.
Total nitrogen..... do	.175	.202	.180	.206	.230	.101	Uncertain.
Loss on ignition..... do	10.33	11.25	8.97	10.06	7.53	3.73	Do.
Reaction..... pH	4.48	4.63	4.69	4.71	4.83	5.02	Positive.
Exchangeable hydrogen ¹ milligram equivalents	10.20	8.92	8.72	9.34	9.46	4.96	Slightly inverse.
Total base capacity ¹ do	16.65	16.56	15.62	16.76	17.04	8.29	None.
Saturation..... percent	38.8	45.8	43.8	43.7	44.4	40.3	Do.
Conductivity.....	9.53	10.22	10.01	10.10	9.79	7.72	Do.
Soluble nutrients per acre by rapid soil tests:							
Calcium..... pounds	10	12	25	26	34	35	Positive.
Phosphorus..... do	1.5	3.2	3.4	3.8	3.6	5.5	Do.
Potassium..... do	35	35	47	41	64	50	Slightly positive.
Ammonia nitrogen..... do	50	58	58	57	75	100	Do.
Aluminum..... do	25	24	25	27	29	14	None.
Iron (ferric)..... do	55	18	22	13	16	8	Slightly inverse.

¹ Per 100 gm. of soil.

Correlations were attempted between soil characteristics and site index of the individual dominant species, considering all plots. This did not prove any more satisfactory than it had where all species were considered together.

This lack of correlation between site index and soil characteristics may be ascribed to other environmental factors which exert an influence on tree growth greater than does the soil. In any forest, soil is only one factor, albeit an important one, in making up the site. It is quite evident from table 6 that other factors must have been operative in some of the plots. Reference to the field notes revealed that on certain plots—11, to be exact—the effect of the soil was obviously overshadowed by other factors. For example, some of the plots on sandy soils that were unquestionably low in fertility had a high site index because of their close proximity to a lake or stream. This meant that the roots had access to an abundant supply of water which favored growth.

Likewise at the other end of the scale, there were plots exhibiting a low site index although the soil sample did not indicate an inferior soil. In some of these cases the soil consisted of a relatively shallow mantle over bedrock; and frequently there was a dense growth of *Vaccinium* or *Gaylussacia* which offered severe competition to the trees. Some plots were located on extremely rocky areas where there was a tendency for the organic matter to accumulate in the crevices between the rocks, and in such cases the soil sample was of little significance. Rockiness in itself does not lower the site quality for forest trees; rather it may improve conditions by favoring aeration, moisture content, and root penetration. Such soils, however, are extremely difficult to sample with any assurance that the sample is representative of the whole soil mass penetrated by the roots.

The presence of any of the afore-mentioned factors is sufficient reason for excluding such plots from consideration so far as soil studies are concerned. Therefore, in table 7 correlations have been attempted between site index and certain soil qualities on 65 plots, omitting the 11 mentioned above. In this instance the groupings by site index were changed somewhat in order to more nearly equalize the number of plots in the several groups.

TABLE 7.—Summary of relation between site index and soil characteristics of 65 plots

Soil characteristic or constituent	Data for site-index class—					Correlation
	40-54	55-59	60-64	65-69	70+	
Average site index.....	50.7	57.7	61.7	67.4	75.8	
Plots.....number.....	11	20	16	11	7	
Total colloids.....percent.....	7.33	6.71	7.13	8.13	6.27	None.
Total sands.....do.....	60.9	63.6	59.1	56.7	64.7	Do.
Moisture equivalent.....do.....	20.6	19.5	21.5	21.7	18.8	Do.
Total nitrogen.....do.....	.158	.178	.206	.215	.220	0.312±.076.
Standard error of mean, σ_m	±.019	±.020	±.017	±.032	±.051	
Loss-on-ignition.....do.....	8.43	8.82	10.41	9.90	9.18	Uncertain.
Reaction.....pH.....	4.74	4.68	4.71	4.71	4.89	None.
Exchangeable hydrogen ¹ , milligram-equivalents.....do.....	7.41	8.84	10.14	8.67	8.95	Do.
Total base capacity ¹do.....	14.12	15.66	17.89	15.80	16.39	Uncertain.
Soluble nutrients per acre by quick test:						
Calcium.....pounds.....	22.3	22.8	24.7	29.1	32.1	Slight.
Potassium.....do.....	46.8	39.9	45.0	40.5	61.4	Uncertain.

¹ Per 100 gm. of soil.

Here it may be observed that the rather definite inverse correlation found in two cases in table 6 has disappeared, and that nitrogen shows a low but significant positive correlation. Otherwise, the results are much the same as in table 6, indicating that on the whole the soil characteristics included in this study are not of first importance in the growth of oaks on the areas in question. It must be recognized, of course, that the relative influence of soil and of environmental factors other than soil may, in some instances, be extremely difficult to evaluate. This appears to be the case in about 25 of the 65 plots. Here there was evidence that soil was not of first importance, although it lacked the certainty of the 11 plots previously mentioned. If, then, the soils of the 40 remaining plots should be compared with their site index a much closer correlation will be found, as follows:

Total nitrogen	$r = 0.717 \pm 0.052$
Moisture equivalent	$r = .674 \pm .058$
Loss on ignition	$r = .629 \pm .064$
Total base capacity	$r = .474 \pm .083$
Total colloids	$r = .095 \pm .106$

Naturally one cannot discard plots at will in order to obtain good correlations. On the other hand, one is equally in error in blindly including all plots regardless of the environmental characteristics of each. Considering the conditions obtaining in these stands, it is evident that, other conditions being equal, the quality of the soil has a definite bearing on the site quality of these oak stands. In previous work in this region, Haig (8) found a fairly good correlation ($r = 0.52$) between site index and the colloidal content of the A horizon in red pine plantations. On the other hand, Hicock et al. (10) found a very poor correlation although they worked in the same general region on the same species.

The results obtained in Finland by Valmari and treated statistically by Ilvessalo (11) show the following correlations between site quality and soil properties:

Loss on ignition	$r = 0.453 \pm 0.078$
Total electrolytes	$r = .407 \pm .081$
Total nitrogen	$r = .736 \pm .056$
Phosphorus	None
Potassium	$r = 0.214 \pm 0.091$
Calcium	$r = .612 \pm .069$

These correlations are in the same order of magnitude as those obtained by the writer on the 40 plots; but since the latter represent only 54 percent of the plots studied, it would appear that in the Connecticut forests the soil is of less significance in determining site quality than it is in the forests of Finland.

RESPONSE OF OAK TO SOIL TREATMENT

It will be of interest at this point to discuss briefly the behavior of red oak under controlled conditions when grown in soil variously treated. In the spring of 1934 a set of 16 concrete soil frames containing Cheshire fine sandy loam which had been fertilized and cropped annually during the preceding 7 or 8 years, was planted to the southern species of red oak (*Quercus rubra* L.). The frames measured 25 by 25 inches with the walls extending about 21 inches below the surface of the soil. There was space for only 5 trees in each frame. The fertilizing materials consisted of urea, applied at the rate of 100 pounds of N per acre; phosphoric acid (H_3PO_4 , 85 percent) at the rate of 200

pounds of P_2O_5 per acre; and potassium acetate ($KC_2H_3O_2$) at the rate of 100 pounds of K_2O per acre. The whips were 6 to 12 inches long when planted, and were then cut back to about 3 inches.

In the spring of 1936 all but one tree was cut off at the ground level and weighed. The results are shown in table 8.

TABLE 8.—Effect of soil treatment on growth of red oak in soil frames at New Haven

Treatment ¹	Green weight per tree of good trees removed in March 1936 ²	Treatment	Green weight of 1 tree April 1937	Treatment	Mean weights of trees in all frames	
					Average per tree	Average per tree when very poor trees are omitted
	Grams		Grams		Grams	Grams
N.....	196	N.....	735	No lime.....	85.1	128
P.....	150	NPK.....	704	Lime.....	64.3	70
NK.....	150	K.....	581	Nitrogen, no lime.....	85.8	144
K.....	127	P.....	490	Nitrogen and lime.....	81.3	84
NP.....	125	NK.....	478	No nitrogen or lime.....	84.5	112
NPK.....	106	PK.....	306	Lime, no nitrogen.....	47.8	56
None.....	100	NP.....	230	No nitrogen.....	66.1	84
PK.....	70	None.....	228	Nitrogen.....	83.5	114
Mean.....	128		469	No phosphorus.....	68.7	93
LNP.....	115	LNP.....	640	Phosphorus.....	80.9	102
LNPK.....	110	LNPK.....	563	No potassium.....	79.3	108
LPK.....	79	LPK.....	547	Potassium.....	70.2	90
LN.....	67	LK.....	429			
LP.....	58	LNK.....	407			
L.....	55	L.....	362			
LNK.....	44	LN.....	337			
LK.....	32	LP.....	331			
Mean.....	70		452			

¹ L=lime.

² All but 1 tree removed March 1936. No data could be obtained on the root systems.

There is no question but that the oaks responded to the different treatments, but the actual order of response is uncertain because of the high degree of variation between individual trees in any one frame. It is believed that the acidity of the soil played an important role in this experiment. When treatments are grouped and averaged, it is seen that the mean growth in the frames receiving lime was lower than in those without lime. The superior showing of nitrogen is partly due to the greater acidity resulting from its use. Lime without nitrogen gave the lowest mean values. Phosphorus was beneficial, but potash was not. The average pH value of the limed series was 5.62; of the unlimed, 4.62.

In contrast, sugar maple (*Acer saccharum* Marshall) planted in an adjoining set of frames identical in every respect, showed a marked response to lime as indicated in table 9. Although the untreated frame was the poorest, neither phosphorus nor potassium showed any consistent beneficial effect.

In a third set of frames white ash (*Fraxinus americana* L.) appeared to be benefited by the presence of lime when all unlimed frames were compared with the limed ones. On the unlimed series all treatments receiving phosphorus, either alone or with nitrogen, were making better growth (in 1938) than those not receiving phosphorus. On the limed series nitrogen seemed to be the most necessary element.

for in all plots receiving nitrogen growth was considerably more rapid than in those without nitrogen. The most reliable data will be obtained, of course, at the conclusion of the experiment when the trees are removed and weighed.

TABLE 9.—*Effect of soil treatment on growth of sugar maple in soil frames at New Haven*

Treatment ¹	Total green weight of 3 trees without leaves or roots	Treatment	Mean weights of trees in all frames
	<i>Grams</i>		<i>Grams</i>
P.....	292	No lime.....	772
LNK.....	289	Lime.....	1,030
LP.....	254	Nitrogen, no lime.....	726
LN.....	252	Nitrogen and lime.....	1,133
NK.....	240	No nitrogen or lime.....	819
LK.....	232	Lime, no nitrogen.....	927
LNP.....	206	No phosphorus.....	904
LNPK.....	198	Phosphorus.....	899
PK.....	175	No potassium.....	909
L.....	163	Potassium.....	894
NP.....	134		
LPK.....	127		
N.....	119		
K.....	116		
NPK.....	113		
None.....	100		

¹ L=lime.

Because of the small number of trees in each frame, the natural variability of individuals so characteristic of hardwoods, and the brief duration of the test, these results are of limited value in ascertaining the nutrient needs of the species in question. Nevertheless, they are useful in indicating trends, and should be so evaluated.

RELATION OF SITE INDEX TO KIND OF SOIL

Thus far only the upper part of the soil profile has been considered. This is the most important part of the mineral soil so far as root feeding and seedling development are concerned and, in the present work at least, it yields the most interesting data when subjected to chemical analysis. Others, including Aaltonen (1), have confined their chemical analyses to samples taken from the first 10 cm. However, the subsoil plays an important role, and in many cases is the controlling influence in determining site quality. On the one hand, a heavy clay subsoil restricting drainage, aeration, and root penetration, and on the other hand, a loose, coarse sand subsoil resulting in excessive droughtiness may be equally deleterious to tree growth. Reference has already been made to the condition in the vicinity of Eberswalde where depth to the underlying marly clay controls the composition and growth of forest and ground vegetation alike. A somewhat similar condition exists at Visingsö, an island in Vättern in south Sweden (18). Also in south Sweden the gravelly kames and eskers have a higher base mineral index than do the sands, and this seems to be correlated directly with site quality.

Previous work (10) has indicated that in this region (Connecticut) several soil series may be biologically equivalent so far as tree growth is concerned. Therefore in attempting to correlate site index with

soil series, it is advisable to combine some of the series into larger groups, ignoring the finer distinctions recognized in soil mapping. In table 10 is shown the distribution of plots by site index and by soil groups. No correlation is indicated, due partly at least to the small representation of some of the soil groups. The high site index of some of the plots in the sandy group is the result of high water table, as previously mentioned.

TABLE 10.—*Relation between site index and kind of soil*

Site-index class	Number of plots of each kind of soil indicated						Total
	Sandy terrace (Merrimac, Enfield)	Upland, red sandstone soils (Cheshire, Wethers- field)	Gravelly (Hinckley, Manchester)	Flat poorly drained (Whitman, Peru)	Upland, friable sub- soil (Glou- cester, Hinsdale, etc.) ¹	Upland, compact subsoil (Charlton, Wood- bridge, Hollis)	
	Number	Number	Number	Number	Number	Number	Number
25-40.....					2		2
40-45.....					2		2
45-50.....	1	1			5	1	8
50-55.....		2	1		5		9
55-60.....	2	2		3	12	2	22
60-65.....	1	1	2	1	7		12
65-70.....		3	1		6	1	12
70-75.....	2	1			2		5
75-80.....		1			1		2
80.....	2						2
Total.....	8	11	5	4	42	6	76

¹ Other series included in this group are: Brookfield, Maltby, and Holyoke.

TABLE 11.—*Comparison of soil groups with respect to certain soil characteristics*

Soil characteristic or constituent	Horizon or depth	Group 1, friable subsoil	Group 2, compact subsoil	Group 3, red sandstone and shales	Group 4, poorly drained	Group 5, gravelly eskers, etc.	Group 6, sandy terrace
Soils in red pine plantations:							
Plots.....number.....		7	5	4		4	3
Total N.....percent.....	A ₁	0.285	0.287	0.236		0.213	0.078
	A ₂	.147	.208	.114		.148	.036
	B	.046	.044	.040		.053	.017
	C	.028	.020	.019		.019	.009
<i>Inches</i>							
Citric acid soluble P.....p. p. m.....	0-6	52	70	32		105	37
	6-18	33	35	15		74	29
Replaceable Ca.....do.....	0-6	125	181	135		109	36
	6-18	93	155	187		76	24
Soils from oak stands:							
Plots.....number.....		144	6	10	4	5	8
Total N.....percent.....	0-5	0.207	0.181	0.220	0.319	0.175	0.088
Exchangeable hydrogen.....milligram equivalents ²	0-5	9.7	8.3	8.1	17.9	9.0	4.9
Exchangeable bases.....do.....	0-5	7.4	6.2	7.9	17.1	6.3	3.6
Total base capacity.....do.....	0-5	17.1	14.5	16.0	34.9	15.3	8.5
Saturation.....percent.....	0-5	42.4	43.1	49.7	48.4	42.5	41.6
Soluble Ca per acre.....pound.....	0-5	23.0	29.0	30.0	19.0	22.0	18.0
Soluble K per acre.....do.....	0-5	42.0	55.0	56.0	31.0	44.0	31.0

¹ Including 5 plots on the Holyoke series.

² Per 100 gm. of soil.

A comparison of some of the characteristics of the several groups of soil series (table 11) shows that the poorly drained soils were outstandingly more fertile than all others, although there were individual soils in groups 1 and 3 that were equally rich. In the pine-

plantation soils the compact subsoil group was richer in some cases than the other groups, but this was not uniformly true nor was it true in the oak soils. No plots were located on limestone soils and only five were on the Holyoke series. The latter is a stony soil derived from a thin glacial deposition on traprock. The traprock influence is sufficient to give these soils a higher base content than the average, but such benefit is overshadowed by soil moisture deficiencies due to the relative thinness of the soil.

RELATION OF SITE INDEX TO HUMUS TYPE

As stated under Description of the Region, the predominating humus types in Connecticut are granular mor and mull. Fibrous mor is encountered only occasionally, as is also firm mull. Within the granular mor group there are many subtypes which should be taken into consideration, but so far no adequate method of evaluation or comparison has been devised. The relation between site index and humus types is shown in table 12.

TABLE 12.—*Relation between site quality and humus type*

ALL PLOTS

Item	Mull	Fibrous mor	Granular mor	Mull-mor	Fibrous mor over podzol
Site index:					
Average.....	63.9	61.6	60.3	59.1	54.8
Range.....	55-76	47-69	48-74	30-83	36-63
Plots.....number..	8	9	33	15	11

AFTER ELIMINATING 4 PLOTS WITH EXTREMELY LOW SITE INDEX

Site index:					
Average.....	63.9	63.0	60.3	61.1	58.3
Range.....	55-76	57-69	48-74	45-83	50-63
Plots.....number..	8	8	33	14	9

AFTER ELIMINATING 13 ABNORMAL PLOTS

Site index:					
Average.....	63.9	60.0	57.5	70.0	54.8
Range.....	55-76	47-67	48-67	54-83	36-63
Plots.....number..	8	8	26	10	11

Considering not only the mean site index for the group, but also the range in site index, it is quite obvious that the mull type ranks first, and the fibrous-mor-over-podzol type last. Among the others there is little choice. By eliminating four plots that were decidedly lower than the others, the resulting means show even smaller differences, as is seen in the second part of table 12. If the 13 plots that are among the "abnormal" group are eliminated, the resulting averages are as shown in the lower part of table 12. This places mull-mor in first place, with mull second. Actually this rating is more nearly correct, for it takes into consideration some of the environmental factors other than soil that affect tree growth.

When the soils under the several humus types are compared the mean values shown in table 13 are obtained. Although individual values varied quite widely in each case, the means show rather def-

inite trends in favor of the mull and mull-mor types. It is of interest to note that of the five plots on Holyoke soil, four were classified as mull or mull-mor types.

From these studies it may be concluded, therefore, that in general good site index is associated with the better humus types.

TABLE 13.—*Relation between soil characteristics and humus type*

Soil characteristic or constituent.	Mull	Mull-mor	Granular mor	Fibrous mor	Fibrous mor over podzol
Total colloids.....percent.....	10.2	9.0	6.5	7.0	5.4
Moisture equivalent.....do.....	22.1	24.3	20.7	20.0	17.8
Total nitrogen.....do.....	195	233	193	172	157
Base capacity.....milligram-equivalents ¹	14.8	16.6	16.5	16.9	14.3
Calcium per acre.....pounds.....	54	38	17	13	13
Potassium per acre.....do.....	70	52	41	37	33

¹ Per 100 gm. of soil.

RELATION OF SITE INDEX TO TOPOGRAPHY

When compared on the basis of topography (table 14) a rather well-defined difference is found between the top and the foot of slopes, the foot, of course, giving the higher site index. No other relationship with topography could be found. Rocky ridges are generally considered unfavorable sites because of the thinness of the soil, the low natural moisture supply, and the greater exposure to drying winds. In this work such sites were not as poor as had been anticipated, although the range in site index was very wide. The data with respect to aspect or exposure were incomplete and therefore cannot be brought into the discussion.

TABLE 14.—*Relation between site quality and topography*

Topography	Plots	Site index	Range	Topography	Plots	Site index	Range
	<i>Number</i>				<i>Number</i>		
Long slope, foot.....	4	67.0	60-73	Rocky ridge, top.....	9	59.1	36-72
Level, well drained.....	18	63.6	47-83	Long slope, top.....	7	57.3	42-76
Level, poorly drained.....	4	60.2	58-63	Short steep slope, middle.....	4	51.0	30-64
Long slope, middle.....	21	60.0	45-74	Short steep slope, top.....	2	50.0	45-55
Moranic deposit, top.....	4	59.5	50-69	Short steep slope, foot.....	1	48.0

RELATION OF SITE INDEX TO COMPOSITION AND RELATIVE ABUNDANCE OF LESSER VEGETATION

Although 82 species were recognized and recorded (in addition to reproduction of tree species) only a few were sufficiently prevalent to warrant an attempt at correlation with site index. In the field the occurrence of each species was estimated and reported as occasional, frequent, abundant, or very abundant. For purposes of correlation, arbitrary values were assigned to each designation and then averaged. Several closely related species or species with similar requirements were lumped together, as follows: the *vacciniums* and *Gaylussacia*, the several *viburnums*, *Rubus* species (*idaeus*, *hispidus*, and *villosus*), the mosses, and the ferns.¹³ The results, shown in table 15, indicate an inverse correlation between site quality and the

¹³ Separation of the ferns according to site preference proved of no advantage and therefore was not made.

vacciniums, the mosses, and to a certain extent, witch-hazel. In the case of *Rubus* species, the correlation is positive as would be expected, for these species are usually found on the better sites. The correlations are more in evidence in the first part of table 15 than they are in the second part where the plots were regrouped in order to give a more equitable distribution of plots.

TABLE 15.—*Relation between site index and presence of lesser vegetation*¹

GROUPED IN SITE-INDEX UNITS OF 10 (FIRST GROUP=20)

Site index	Plots	Relative abundance of—						
		<i>Vaccinium, Gaylussacia</i>	<i>Kalmia latifolia</i>	<i>Hama- melis vir- giniana</i>	<i>Viburnum</i>	<i>Rubus spp.</i>	Mosses	Ferns
	Num- ber							
20-39.....	2	80	0	15	20	0	30	25
40-49.....	8	33	10	15	9	0	20	9
50-59.....	28	44	3	10	17	1	14	14
60-69.....	26	33	14	12	19	3	10	19
70-79.....	7	11	1	8	7	6	13	17
80+.....	2	0	0	0	0	5	5	0

GROUPED IN SITE-INDEX UNITS OF 5 (EXCEPT FIRST AND LAST GROUPS) IN ORDER TO OBTAIN MORE EQUITABLE DISTRIBUTION

20-49.....	11	42	8	14	0	0	20	11
50-54.....	9	44	7	0	28	0	13	20
55-59.....	18	45	1	16	13	1	15	12
60-64.....	16	39	13	10	14	4	11	18
65-69.....	8	30	10	11	25	0	5	18
70+.....	12	12	8	10	10	7	12	15

¹ Arbitrary values used for purposes of averaging: Very abundant, 80; abundant, 50; frequent, 30; occasional, 10.

RELATION OF KIND OF SOIL TO OCCURRENCE OF LESSER VEGETATION

Using the soil groupings as given in table 10, the writer has listed the "relative occurrence" of the reproduction of tree species in Table 16 and that of the lesser vegetation in table 17. By relative occurrence is meant the relative abundance and frequency of a species in relation to the number of plots in any particular soil group. It was determined as follows: As shown in table 15, arbitrary values were assigned to each estimate of abundance; viz, Very abundant=80, abundant=50, frequent=30, and occasional=10. The sum of these values for each species on each soil group was divided by the number of plots of that particular soil group, giving the relative occurrence. For example,

Amelanchier spp. on gravelly soil:

Abundance values—10, 10, 30, 10. Sum=60

Number of plots on gravelly soil=5. $60 \div 5 = 12$ relative occurrence. By this calculation it was possible to compare soil groups with each other even though no two groups had the same number of plots.

Although the relatively small number of plots in most of the soil groups restricts the reliability of the results, these are nevertheless, of considerable interest. According to the data in table 16, gray birch, red maple, and especially oak reproduction was quite abundant on the sandy soils. Black cherry and sassafras were found in greater abundance on the heavy subsoils than any of the other species. No

reproduction was found on the poorly drained soils. All species reproduced on the loose subsoil type represented by the Gloucester and closely related soil series.

TABLE 16.—Relative occurrence ¹ of reproduction of tree species on different types of soil

Species	Relative occurrence—				
	Compact subsoil	Red sand-stone soils	Loose subsoil	Gravelly soil	Sandy soil
Ash	1.6		2.1		
Birch, black			1.6		
Birch, gray			2.1		7.5
Cherry, black	6.7	6.4	9.5	6	
Hemlock		2.7	.5	2	
Maple, red	3.3	3.6	13.8	10	8.8
Maple, sugar	3.3		3.1		
Oak	1.2	11.8	20.7	16	21.2
Pine, pitch			.5		
Pine, white			1.4	4	1.3
Sassafras	5.0	2.7	6.4	2	1.3

¹ Relative occurrence = $\frac{\text{sum of occurrence}}{\text{Number of plots in the soil group}}$. No reproduction on poorly drained soil.

TABLE 17.—Relative occurrence ¹ of lesser vegetation on different types of soil

[Number of plots in parentheses]

Species	Relative occurrence on—					
	Poorly drained	Compact subsoil	Red sand-stone soils	Loose sub-soil	Gravelly soil	Sandy soil
<i>Amelanchier</i> spp.		1.6 (1)	1.0 (1)	6.2 (10)	12 (4)	6.3 (3)
<i>Amphicarpa monoica</i>				1.9 (4)	6 (1)	3.8 (1)
<i>Aralia</i> spp.				2.6 (7)	6 (1)	2.5 (2)
<i>Aster</i> spp.			7.3 (4)	3.3 (6)		
<i>Benzoin aestivale</i>	10 (1)			.7 (1)		
<i>Carpinus caroliniana</i>	10 (1)	1.6 (1)		.3 (1)		
<i>Chimaphila maculata</i>		1.6 (1)		1.9 (4)		6.3 (3)
<i>Chimaphila umbellata</i>		1.6 (1)		5.0 (9)	4 (2)	3.8 (1)
<i>Clethra alnifolia</i>	20 (2)			.7 (1)		
<i>Cornus alternifolia</i> and <i>paniculata</i>		1.6 (1)	3.6 (2)	.3 (1)	2 (1)	
<i>Cornus florida</i>		10.0 (2)	10.9 (4)	12.1 (15)	8 (2)	
<i>Corylus americana</i>			3.6 (2)	3.3 (6)	2 (1)	10.0 (4)
<i>Crataegus</i> spp.		3.2 (2)		1.4 (2)		
Ferns	30 (3)		25.4 (12)	26.2 (32)	8 (2)	5.0 (2)
<i>Gaultheria procumbens</i>			1.0 (1)	5.2 (8)	6 (1)	7.5 (2)
Gramineae		16.6 (4)	20.9 (9)	24.2 (35)	30 (5)	18.3 (7)
<i>Hamamelis virginiana</i>		5.0 (1)	8.2 (3)	13.6 (17)		
<i>Kalmia angustifolia</i>			2.7 (1)	1.9 (4)		5.0 (2)
<i>Kalmia latifolia</i>			5.4 (2)	10.5 (14)	2 (1)	1.2 (1)
<i>Lycopodium</i>				.5 (2)	2 (1)	1.2 (1)
<i>Maianthemum</i>			2.7 (1)	.7 (3)		
<i>Mitchella repens</i>		3.2 (2)	3.6 (2)	2.4 (6)	2 (1)	
Mosses		1.6 (1)	7.3 (4)	17.4 (20)	10 (3)	11.2 (5)
<i>Myrica asplenifolia</i>				.5 (2)		1.2 (1)
<i>Ostrya virginiana</i>		1.6 (1)	4.5 (1)	4.8 (8)	4 (2)	
<i>Pyrola</i> spp.		1.6 (1)		1.0 (2)		2.5 (2)
<i>Pyrus arbutifolia</i>			1.0 (1)	1.4 (2)		
<i>Rhododendron canescens</i>		5.0 (1)	5.5 (2)	6.4 (11)		
<i>Rosa</i> spp.		1.6 (1)				1.2 (1)
<i>Rubus</i> spp.		3.3 (2)		2.9 (6)		3.8 (3)
<i>Smilacina</i> spp.				2.9 (4)		
<i>Smilax</i> spp.				2.9 (4)	2 (1)	
<i>Vaccinium</i> spp. and <i>Gaylussacia</i> spp.	20 (2)	40.0 (7)	34.5 (14)	72.4 (69)	60 (8)	37.5 (6)
<i>Viburnum acerifolium</i>		3.3 (2)	17.3 (7)	19.0 (26)	4 (2)	7.5 (2)
<i>Viburnum cassinoides</i> , <i>dentatum</i> and <i>lentago</i>	20 (2)		4.5 (3)	.3 (1)		

¹ Relative occurrence = $\frac{\text{Sum of occurrence}}{\text{Number of plots in the soil group}}$

When it comes to the lesser vegetation exclusive of tree seedlings, (table 17), six species or species groups occurred on poorly drained soil. *Vaccinium* and/or *Gaylussacia* were found on all soil types but tended to be in greater abundance on the drier soils. Ferns were found on all except the compact subsoil group. Grasses show a quite uniform distribution on all except wet soils. The failure to find on either gravelly or sandy soils *Aster*, *Benzoin*, *Carpinus*, *Clethra*, *Crataegus*, *Hamelis*, *Maianthemum*, *Pyrus*, *Rhododendron*, *Smilacina*, and all species of *Viburnum* except *acerifolium* may or may not be significant. Practically all of these species are known, however, to prefer moist situations.

DISCUSSION

From the literature we learn that, by and large, the chief limiting soil factors in forests at moderate elevations in the Temperate Zone are soil moisture, lime content, and porosity or aeration of the soil. In some sections one factor predominates, in other sections another. In Connecticut with its light soils, porosity or soil aeration is not a factor (except in strictly local situations). Lime and other bases are of importance for the more exacting hardwood species; but lime alone has relatively little effect on the conifers and for the most part on the oaks. This was demonstrated for oak in the soil frame experiment previously described. In other words, a low lime content cannot be said to be a limiting factor in oak stands in Connecticut.

In certain situations where an undesirable humus condition is caused by or maintained through the presence of *Vaccinium* and *Gaylussacia*, a higher base content in the soil would probably favor the presence of more desirable shrub and ground-cover species and thus benefit the oaks indirectly. Furthermore, *Vaccinium* and *Gaylussacia* offer severe competition to the oaks, and their elimination would be beneficial. The difficulty in establishing pine on the heath lands of northwestern Germany and Denmark is well known. Wiedermann (22) states that berry bushes (blueberry and huckleberry) growing under a good tree species frequently exert an unfavorable influence on the soil; whereas a raspberry or grass flora may bring about favorable conditions even under a poor species.

Stålfelt (17) reports that the loss of water from a soil with a shrub cover is much greater than from a bare soil or from one covered only with forest litter or with moss. Wittich (23) states that on the sandy soils of north Germany, bare soil has a lower water content up to about June 1 than soil supporting shrub growth; but from then on into autumn the bare soil is the more moist.

Although this study did not include measurements on soil moisture, it was obvious in many situations that moisture is a very important factor. While the ideal plan for such studies would require frequent measurements throughout the year, such a program is not always feasible. The determination of the relative moisture content during the driest and hottest part of the summer is a good substitute, and such determinations were made in 1934 and 1935 in a number of forests, but without reference to the particular oak stands discussed in this paper and without growth measurements. The field moisture content was compared with the moisture equivalent to give the relative wetness $\frac{\text{field moisture}}{\text{moisture equivalent}} \times 100 = R.W.$ Some of the results are given in table 18.

TABLE 18.—*Relation between apparent site quality, and relative wetness ¹ of some forest soils during the driest part of the summer*[Number of samples ² in parentheses]

Location and kind of forest	Apparent site quality	1934, 0-5 inches	1935	
			0-5 inches	12-15 inches
Meshomasic Forest, Cox area, mixed hardwoods:				
Upper slopes and ridges.....	Fair to poor.....	96(1)	77(1)	66
Middle slopes (gentle).....	Medium.....	91(1)	123(3)	122
Lower slopes.....	Good.....	166(3)	166(3)	139
Meshomasic Forest, mountain top, scarlet oak.....	Poor.....	107	-----	-----
Meshomasic Forest, upper slope, mixed hardwoods ³	Medium.....	117	-----	-----
Mount Carmel, hilltop, mixed hardwoods.....	Fair.....	66(1)	55(1)	55
Mount Carmel, lower slope, mixed hardwoods.....	Good.....	82(1)	61(1)	47
Rainbow plantation, Merrimac coarse sand:				
Red pine check plot.....	Poor.....	58	47	46
Red pine raked plot ⁴	do.....	52	41	44
Red pine triple-thickness plot ⁵	do.....	58	54	47
Oak plot ⁶	do.....	49	65	67

¹ Relative wetness = $\frac{\text{field moisture content}}{\text{moisture equivalent}} \times 100$.

² Each sample represents from 2 to 4 borings.

³ Red oak, scarlet oak, white oak, red maple.

⁴ Soil raked clear of all organic debris every fall.

⁵ Litter added yearly from 2 raked plots, making the A_o horizon 3 times its normal thickness.

⁶ Rather open stand of red oak, scarlet oak, and white oak, all 6-14-inch d. b. h., underplanted with white pine 2-3-feet tall.

Owing to the inequalities in precipitation, especially in summer, it is not safe to compare one forest or locality with another without having a rain gage in each. But within any one locality comparisons are possible, and they are valuable in indicating the soil moisture conditions during the most critical period. These data show that a more or less direct correlation exists between the apparent site quality and the relative wetness of the soil, and they lend support to the belief that moisture is undoubtedly the chief limiting soil factor in tree growth in Connecticut in spite of the relatively abundant rainfall that is quite uniformly distributed throughout the year. It is difficult, of course, to separate moisture per se from its accompanying effects: viz, favorable humus condition, good microbiological and macrobiological activity, and availability of soil nutrients.

Excess moisture, on the other hand, results in limitation of root development, reduction of ferric iron, nitrates, and other compounds, and cessation of biological activity. Morgan ¹⁴ when investigating the cause of a number of deaths of young trees in an oak plantation in New Jersey, found a very high content of ferrous iron in the soil surrounding the roots of the dead trees. He attributed this to the fact that a considerable quantity of organic matter in the form of sod had been incorporated in the soil at the time of planting, and the wet soil with resulting poor aeration caused a reduction of some of the iron to the ferrous condition. In the case of the living trees, little or no sod got into the planting hole.

Soil tests reveal that the average forest soil in Connecticut is more acid and is considerably lower in fertility than the average farm soil as shown in table 19.

With respect to soil and humus types, it is of interest to note that scarlet oak was the predominant species on 9 out of 11 plots showing evidences of podzolization. Nine of the eleven were on the Gloucester

¹⁴ MORGAN, M. F. Private communication.

ter-Hinsdale group of soils, 1 on gravelly soil (Hinckley) and 1 on wind-blown soil (Enfield). On 10 of these plots huckleberry and lowbush blueberry, especially the former, were by far the most abundant element in the ground cover. Accompanying shrubs were mostly ericads (*Kalmia latifolia*, *K. angustifolia*, *Vaccinium corymbosum*, etc.). Bracken fern was generally common also.

TABLE 19.—*Comparison of soluble nutrients in typical forest, farm, and greenhouse soils by Morgan's rapid soil test*¹

Soluble nutrient	Forest soil, pH 4.5	Farm soil, pH 5.6	Greenhouse soil, pH 6.5	Soluble nutrient	Forest soil, pH 4.5	Farm soil, pH 5.6	Greenhouse soil, pH 6.5
	<i>Pounds per acre</i>	<i>Pounds per acre</i>	<i>Pounds per acre</i>		<i>Pounds per acre</i>	<i>Pounds per acre</i>	<i>Pounds per acre</i>
Nitric nitrogen.....	1	25	90	Potassium.....	75	200	500
Ammonia nitrogen.....	20	15	50	Calcium.....	500	3,000	4,500
Phosphorus.....	25	100	250	Aluminum.....	200	50	50

¹The reader is cautioned not to compare the above results with those given for rapid soil tests in table 6. As previously explained, changes in procedure and color scales about 1934 so altered the readings obtained that direct comparison is not possible.

Only one pure scarlet oak stand was associated with a mull condition, and that was a young plantation on a moist and somewhat heavier agricultural soil (Wethersfield). No stand of predominantly red oak was associated with anything poorer than granular mor. The ground cover found on mull and mull-mor was typically *Viburnum acerifolium*, *Cornus florida*, and a great variety of smaller shrubs, wild flowers, and herbs; but *Gaylussacia* and *Vaccinium* spp. are generally present and often abundant. *Viburnum*, *Cornus*, shrubs, wild flowers, and herbs were conspicuously absent on the fibrous-mor-over-podzol plots where the ericads predominated.

SUMMARY

Seventy-six temporary plots in even-aged second-growth oak stands well distributed throughout the State of Connecticut furnish the basis for this study. Height, diameter at breast height, and age of the dominant and codominant oaks were ascertained separately for each species. The five principal species were white, red, black, scarlet, and chestnut oak, and there were a few swamp white and pin oak. Notes were taken on lesser vegetation, topography, and soil; and the soil was sampled to a depth of about 5 inches.

Site indices were calculated from height curves and formulas supplied by the Forest Service of the United States Department of Agriculture. In the absence of proof to the contrary, it was assumed that the Forest Service curves were applicable to the Connecticut data.

The climate of Connecticut is sufficiently warm and humid to favor tree growth. The soils are prevalingly acidic, largely fine sandy loam in texture, and relatively low in fertility.

The results of this study may be summarized as follows:

Site indices ranged from 30 to 83 and averaged 59.7. Nearly three-quarters of the plots had a site index between 50 and 70, and slightly over three-quarters were between the ages of 20 and 60 years. Black oak averaged highest in site index, and chestnut oak lowest. The average height and diameter at breast height of all trees was 53.2 feet and 7.98 inches, respectively.

When all plots were included, correlations between site index and characteristics of the surface soil were, for the most part, either entirely absent or slightly inverse. In some cases it was obvious that other environmental factors, such as high water table or seepage from higher ground, were of greater consequence than the soil itself in controlling tree growth. The omission of 11 plots that exhibited obvious external influences resulted in the elimination of the inverse correlations mentioned above, and at the same time revealed a low but significant positive correlation between site index and total nitrogen. This indicated that, taking the stands as they were, the soil was not of first importance in the growth of oaks. On the other hand, where conditions are otherwise similar, the quality of the upper A horizon of the soil is more or less directly correlated with the site quality of these oak stands.

Soil series groups showed no correlation with site index.

With respect to humus types, plots having a mull condition averaged the highest site index, and the fibrous-mor-over-podzol type, the lowest.

Topographically, there was little correlation other than the obvious one, that plots at the foot of a slope have an average site index considerably greater than those on hilltops. Level, well-drained areas were slightly superior to level, poorly drained areas.

Site index was inversely correlated with the presence of *Vaccinium* and *Gaylussacia*, the mosses, and, to a certain extent, witch-hazel; but there was evidence of a direct correlation with the presence of *Rubus*.

No reproduction of tree species was observed on the poorly drained soils. On sandy soils, gray birch, red maple, and oak were fairly abundant; on the compact subsoil group black cherry and sassafras were in greater abundance than most of the other species.

Shrubs and herbaceous plants were found pretty well in accordance with their preferred environment, based largely on water relations.

With respect to vegetation and soil condition, the fibrous-mor-over-podzol type was characterized by a predominance of scarlet oak in the stand, and huckleberry and blueberry in the ground cover. On the mull type no one species of oak predominated; but the ground cover was typically *Viburnum acerifolium*, *Cornus florida*, and numerous smaller shrubs, wild flowers, and herbs, and in addition some blueberry and huckleberry.

CONCLUSIONS

In the second-growth oak stands of Connecticut site quality is the resultant of a number of environmental factors, chief of which are those affecting the water economy of the tree; viz, available moisture, topography, and depth of soil. It is apparent that the native fertility of the soil is an important determining factor in *stand composition* (as evidenced by the fact that only the less exacting species can survive on the poorer soils); but the fertility of the soil as measured by the tests used in this study is a controlling factor in *site quality* only in those situations where other environmental influences are of minor significance. Stated another way, when other conditions are equal, soil fertility is a controlling influence in determining the site quality of oak stands in Connecticut.

Topography comes into the picture only as it influences soil-moisture supply and exposure to or protection from wind and sun.

Except for a few species, the ground cover in these oak stands appears to be of little value in indicating site quality.

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HOST RANGE STUDIES WITH BACTERIUM SOLANACEARUM ¹

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INTRODUCTION

Bacterium solanacearum E. F. Smith ³ is the cause of a wilt disease of tobacco (*Nicotiana tabacum* L.), potato (*Solanum tuberosum* L.), tomato (*Lycopersicon esculentum* Mill.), redpepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), banana (*Musa sapientum* L.), peanut (*Arachis hypogaea* L.), and a number of ornamental and wild plants. The disease on tobacco is known in the United States as Granville wilt, which is severe in certain areas of North Carolina and adjoining States. Crop rotation is the major control measure for the disease. Inasmuch as both wild and cultivated plants are known to be susceptible, the host range of the causal organism is an important factor in its control. The principal object of this investigation was to determine the susceptibility of the crops and field weeds of the wilt-infested area to natural infection. This information is pertinent to the crop-rotation experiments in progress at the present time.

Successful stem inoculation has been accepted as proof of susceptibility to the disease. However, several species on which stem inoculation has been successfully carried out were not known to become diseased when grown on infested soil, even though certain of these species are widely grown as crop plants. It appeared that certain species might be susceptible to artificial stem inoculation and yet not become naturally diseased by infection through the roots from the soil-borne parasite. This possibility made it necessary to compare the susceptibility of plants to natural and artificial infection. The experiments were in progress during 1935, 1936, 1937, and 1938.

The literature on *Bacterium solanacearum* has been reviewed by Palm and Jochems (13), ⁴ Elliot (4), and Labrousse (12). The reader is referred to these publications for world-wide host lists and bibliographies. Host range studies applicable specifically to Granville wilt have been conducted by Fulton and Winston (6), Fulton and Stanford (5), Stanford and Wolf (20), and Wolf (22).

METHODS

All species under test were grown in rows for one or more seasons on soil known to be very highly infested with *Bacterium solanacearum*.

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² The writer is indebted to Dr. R. F. Poole, of the North Carolina State College, E. G. Moss, of the Tobacco Branch Station, and Dr. E. E. Clayton, of the Division of Tobacco and Plant Nutrition, for suggestions and criticisms, and also to Dr. M. F. Buell, of the college, and R. K. Godfrey, formerly of the Division of Tobacco and Plant Nutrition, for aid in the identification of the plants.

³ *Phytophthora solanacearum* (E. F. Smith) Bergey et al.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 439.

Cultivation with horse-drawn and hand implements encouraged natural infection after root injury. In order to measure the severity of wilt during each season, every third or fifth row of the host range plantings was set with varieties of tobacco known to be highly susceptible. Figure 1 is a field scene illustrating the severe disease conditions under which the tests were conducted. In taking final notes on susceptibility to natural infection, counts were made of plants showing symptoms on the below-ground as well as on the above-ground parts. The results of the natural-infection tests have been



FIGURE 1.—Field scene illustrating arrangement of tobacco checks and severity of wilt on naturally infested field used for the experiments. *a*, Tobacco, 95-percent diseased; *b*, red pepper, 50-percent diseased; *c*, tomato, 100-percent diseased; *d*, tobacco, 92-percent diseased. Photographed August 7, 1935.

supplemented by hundreds of observations made on plants growing on wilt-infested farms.

Tests of susceptibility to inoculation were usually made on 5 to 10 individuals in the plantings described in the previous paragraph. Inoculations were made by inserting small wedges of discolored tissue from the woody cylinder of diseased tobacco plants into the stems of the plants just below the terminal buds. Where possible, inoculations were made on small plants during early summer, a period during which wilt is quite active in the field. Infected tissue used immediately after its removal from the plants provided a constant source of virulent inoculum. Pure cultures were not satisfactory because *Bacterium solanacearum* varies in pathogenicity when maintained in artificial culture.

Bacterium solanacearum was proved from all infected species. Tissue inoculations were made from the diseased plants into tobacco

growing on disease-free soil for all known host plants showing typical wilt symptoms. Tobacco for these confirmatory inoculations was grown on Cecil or Iredell sandy loam soils not previously cropped to tobacco and on which the disease is not generally present. More specific proof of *Bact. solanacearum* was required for all new or questionable hosts. Pure-culture isolation and needle-prick inoculations were made into tobacco for these species. In addition, laboratory tests for gas production on glucose broth, nitrate reduction, gelatin

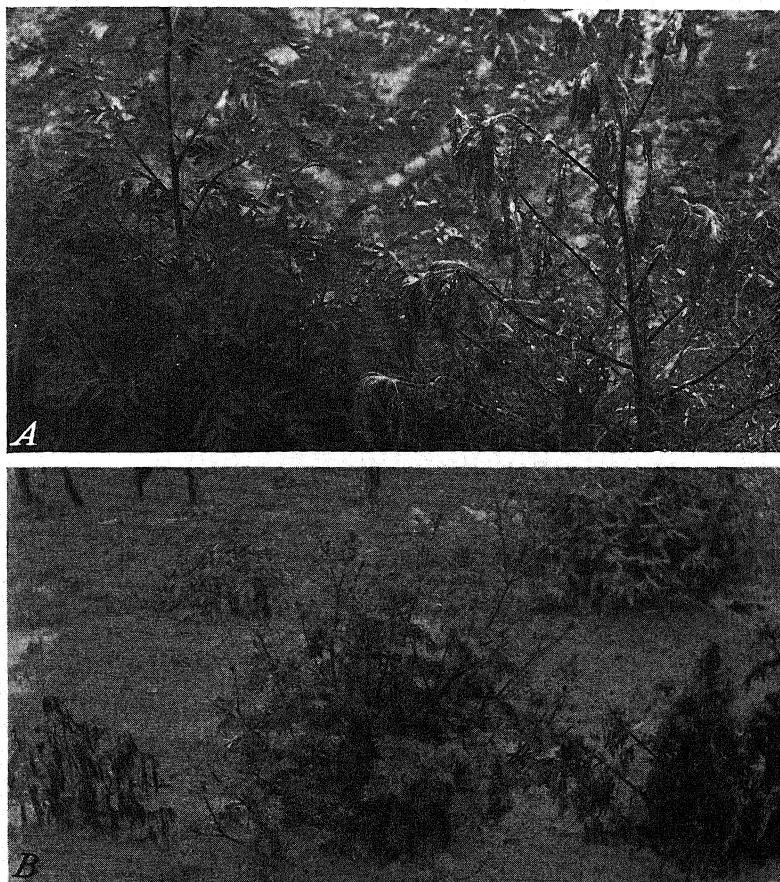


FIGURE 2.—Granville wilt on weeds from natural infection. A, Common ragweed (*Ambrosia elatior* L.), a common field weed of slight susceptibility; healthy plant on left, diseased plant on right. B, Spanish-needles (*Bidens bipinnata* L.), which became 100-percent diseased in all plantings; healthy plant in center, diseased plants on left and right.

liquefaction, and pigment production on steamed potato cylinders were carried out. The last-named test was especially useful in distinguishing *Bact. solanacearum* in the laboratory. A characteristic brownish-black pigment is produced within a few days after inoculation on this medium.

RESULTS

In discussing the susceptibility of the different species to the wilt disease it is convenient to divide them into three classes: Class 1, species that are highly and definitely susceptible, and hence host plants; class 2, the species about which the results raise a question as to whether they should be regarded as hosts; and class 3, species that are definitely immune.

SUSCEPTIBLE SPECIES

The species reacting positively to both natural-infection and stem-inoculation tests were considered susceptible. These are listed in table 1, together with the type of infection observed by the original investigator for each host, and reference to the first literature citation. For convenience four susceptibility classes are given. These are defined in the table.

TABLE 1.—*Species susceptible to natural and artificial infection by Bacterium solanacearum*

Botanical name	Common name	Type of infection observed by original investigator ¹	Approximate number of plants grown	Susceptibility to natural infection
<i>Nicotiana tabacum</i> L.....	Tobacco.....	I (10).....	1,000	High (71 to 100 percent diseased).
<i>Lycopersicon esculentum</i> Mill.....	Tomato.....	N (17).....	51	Do.
<i>Nicotiana rustica</i> L.....	Aztec tobacco.....	N (9).....	89	Do.
<i>Solanum melongena</i> L.....	Eggplant.....	N (16).....	102	Do.
<i>Datura stramonium</i> L.....	Jimsonweed.....	I (16).....	147	Do.
<i>Solanum nigrum</i> L.....	Black nightshade.....	I (16).....	129	Do.
<i>Croton glandulosus</i> L.....	Croton.....	I (20).....	24	Do.
<i>Bidens bipinnata</i> L.....	Spanish-needles.....	I (20).....	49	Do.
<i>Xanthium pennsylvanicum</i> Wallr.....	Cocklebur.....	9	Do.
<i>Tropaeolum minus</i> L.....	Nasturtium.....	N (1).....	58	Do.
<i>Solanum tuberosum</i> L.....	Potato.....	N (16).....	84	Do.
<i>Eclipta alba</i> (L.) Hassk.....	N (5).....	11	Do.
<i>Capsicum annuum</i> L.....	Red pepper.....	N (8).....	85	Moderate (51 to 70 percent diseased).
<i>Physalis pruinosa</i> L.....	Ground cherry.....	29	Do.
<i>Helianthus annuus</i> L.....	Sunflower.....	I (18).....	87	Do.
<i>Phaseolus vulgaris</i> L.....	String bean.....	N (19).....	153	Slight (21 to 50 percent diseased).
<i>Ambrosia elatior</i> L.....	Common ragweed.....	N (5).....	148	Do.
<i>Aster pilosus</i> Willd.....	Aster.....	13	Do.
<i>Dahlia rosea</i> Cav.....	Dahlia.....	N (22).....	77	Do.
<i>Ricinus communis</i> L.....	Castor-bean.....	N (18).....	117	Do.
<i>Tagetes erecta</i> L.....	Marigold.....	N (14).....	73	Do.
<i>Petunia hybrida</i> Vilm.....	Petunia.....	N (16).....	17	Do.
<i>Arachis hypogaea</i> L.....	Peanut.....	N (18).....	121	Very slight (1 to 20 percent diseased).
<i>Leptilon canadense</i> (L.) Britton.....	Horseweed.....	I (20).....	129	Do.
<i>Ambrosia trifida</i> L.....	Giant ragweed.....	40	Do.
<i>Solanum carolinense</i> L.....	Horsenettle.....	I (20).....	89	Do.
<i>Xanthium chinense</i> Mill.....	Cocklebur.....	9	Do.
<i>Verbena hybrida</i> Hort.....	Verbena.....	I (1).....	36	Do.
<i>Cosmos bipinnatus</i> Cav.....	Cosmos.....	N (22).....	94	Do.

¹ I=reported susceptible in the literature on the basis of stem inoculation only; N=reported susceptible in the literature on the basis of observed natural infection.

Twenty-nine species were susceptible. Of these, tobacco, tomato, *Nicotiana rustica*, eggplant, Spanish-needles (fig. 2, B), jimsonweed, black nightshade, croton, one species of cocklebur (*Xanthium pennsylvanicum* Wallr.) (fig. 3), and nasturtium are very highly susceptible, becoming 100 percent diseased in all plantings. Tomato,

black nightshade, and Spanish-needles were the most susceptible, usually becoming 100 percent diseased 2 weeks earlier than adjacent plantings of tobacco.

Redpepper (fig. 1) and other species were much more tolerant. Root rot rather than the foliar wilting was usually the predominant symptom on the less susceptible species. This was especially true of castor-bean, horseweed, ragweed, and horsenettle.

Wide variation in disease percentages for cocklebur during different seasons and from field to field during the same year was at first quite puzzling. Later it was discovered that at least two species were present in the area. These were planted on highly infested soil in 1938. *Xanthium pennsylvanicum* was very highly susceptible and *X. chinense* was only slightly susceptible (fig. 3). This variation is of interest because the highly susceptible species is not of widespread occurrence in the wilt-infested area, while the less susceptible species



FIGURE 3.—Variation in susceptibility of *Xanthium* species to Granville wilt by natural infection: a, *Xanthium pennsylvanicum* Wallr., very highly susceptible; b, *X. chinense* Mill., slightly susceptible.

is generally present. Five species of native weeds are reported here for the first time as new hosts. These are *Xanthium pennsylvanicum*, *X. chinense*, *Physalis pruinosa*, *Aster pilosus*, and *Ambrosia trifida*.

SPECIES SUSCEPTIBLE TO ARTIFICIAL STEM INOCULATION AND APPARENTLY IMMUNE TO NATURAL INFECTION

Each species listed in table 2 was consistently susceptible to artificial stem inoculation throughout these experiments. On the other hand, they apparently never became naturally diseased by infection through the roots from the soil-borne parasite. The fact that cowpeas, soybeans, and velvetbeans were susceptible to inoculation is especially interesting, since they are important summer legumes and are often grown on tobacco land. Detailed studies were restricted to these latter species and consisted of extensive stem-inoculation and natural-infection tests on several varieties of each species and measurements

of the population of *Bacterium solanacearum* established in these plants following stem inoculation.

TABLE 2.—*Species susceptible to artificial infection and immune to natural infection by Bacterium solanacearum*

Botanical name	Common name	Type of infection observed by original investigator ¹	Approximate number of plants grown	Susceptibility to natural infection
<i>Vigna sinensis</i> Endl.....	Cowpea.....	I (19)..... N (15).....	770	Immune (none diseased).
<i>Soja max</i> (L.) Piper.....	Soybean.....	I (19)..... N (22).....	760	Do.
<i>Stizolobium deeringianum</i> Bort.	Velvetbean.....	I (20).....	605	Do.
<i>Phaseolus lunatus</i> L.....	Lima bean.....	I (19).....	175	Do.
<i>Canna</i> sp.....	Canna.....		9	Do.

¹ I=reported susceptible in the literature on basis of stem inoculation only; N=reported susceptible in the literature on the basis of observed natural infection.

Field and greenhouse plantings were made of various varieties ⁵ of cowpeas, soybeans, and velvetbeans. All plantings remained free of natural infection as far as could be detected on both above- and below-ground parts (fig. 4). Inoculations made in the greenhouse

⁵ Cowpeas: Victor, Brabham, Black, Iron, Whippoerwill, and New Era. Soybeans: Otoctan, Mammoth Yellow, Laredo, Tokyo, Biloxi, and Virginia. Velvetbeans: Osceola, Hundred-Day Speckled, Bush, Early Arlington, and Tracy Black.



FIGURE 4.—Field plantings of summer legumes with checkrows of tobacco: *a*, Tobacco; *b*, cowpeas; *c*, soybeans; *d*, tobacco; *e*, velvetbeans. The legumes were susceptible to inoculation but remained free of wilt from natural infection when grown on soil where the adjacent checks of tobacco were, respectively, 95- and 96-percent diseased on August 1, 1935.

with pure cultures of *Bacterium solanacearum* showed that seedlings of all varieties of these three species were susceptible and that the velvet-bean varieties as a group were the most susceptible. However, seedlings of all species were killed by the disease. The plants grown in the field were hardier and hence less susceptible to inoculation. Under field conditions the symptoms were always localized. They consisted of wilting and bacterial occupation of the xylem vessels adjacent to the point of inoculation for all varieties except the Biloxi soybean. In both greenhouse and field inoculations (fig. 5, A) plants

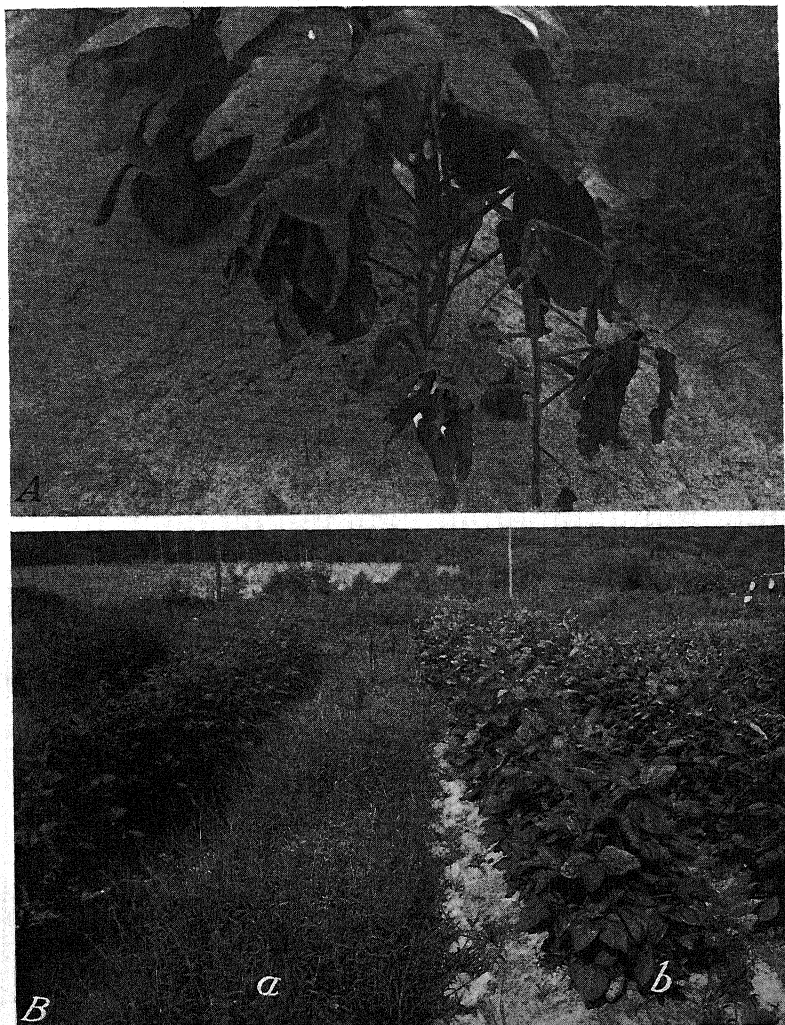


FIGURE 5.—Inoculation and natural-infection tests with the Biloxi soybean. Plants of this variety were killed by the disease or developed localized symptoms following stem inoculation (A) but were immune when grown, (B, b) on soil so highly infested with *Bacterium solanacearum* that tobacco (B, a) was 100-percent dead by August 1, 1937.

of this variety were killed by the disease. However, no evidence of natural infection was found when the Biloxi variety was grown during 2 separate years on soil so highly infested with *Bact. solanacearum* that the adjacent tobacco checkrow was 100 percent dead by August 1 of each year (fig. 5, B).

These results confirm the work of Smith and McCulloch (19) and Stanford and Wolf (20) with cowpeas, soybeans, and velvetbeans for the stem-inoculation tests. However, the reports of natural infection on cowpeas by Schwarz (15) and on soybeans by Wolf (22) were not confirmed. No inoculations were reported by either author. It is doubtful whether the identity of the organism could be definitely established by its appearance in culture alone. Also, since there are various wilt diseases of cowpeas and soybeans caused by micro-organisms and insects, the occurrence of wilted plants is not necessarily an indicator of *Bacterium solanacearum*.

POPULATIONS OF "BACTERIUM SOLANACEARUM" IN THE LEGUMES FOLLOWING STEM INOCULATION

Since cowpeas, soybeans, and velvetbeans were immune to natural infection, it was of interest to determine the size of the bacterial populations established in these plants following stem inoculations made the latter part of June in 1936 and 1937. For this determination, dilution plates were poured from samples of infested areas in the stem. Uniform amounts of tissue were taken 1 inch below the point of inoculation because the organism spreads more rapidly down than up the stem. The 1936 samples were taken 2 weeks after inoculation and the 1937 samples 5 weeks after inoculation. In each series an isolation from naturally infected tobacco was made for comparison. The bacterial population of diseased tobacco tissues should represent the population level reached by *Bacterium solanacearum* in a susceptible plant. All isolations were made from field material.

The data for the counts, which are summarized in table 3, show that after stem inoculation a very small population of *Bacterium solanacearum* in proportion to that of naturally infected tobacco became established in the resistant plants. The wide difference between the bacterial populations of tobacco and the legumes indicates that the latter are not well adapted as food plants for this organism. Nevertheless it is able to persist for at least 5 weeks, but with the exception of the Biloxi variety of soybean (fig. 5) the population apparently never became large enough to cause widespread pathological symptoms.

TABLE 3.—Populations of *Bacterium solanacearum* in inoculated stems of cowpeas, soybeans, and velvetbeans

Plant species	Isolations made		Bacteria at uniform dilution	
	1936	1937	1936	1937
	Number	Number	Number ¹	Number ¹
Tobacco.....	1	1	874,000	190,000,000
Cowpea.....	5	3	30,000	711,666
Soybean.....	2	3	12,000	761,666
Velvetbean.....	3	3	35,000	19,666

¹ Average.

IMMUNE SPECIES

The species reacting negatively to both inoculation and natural-infection tests were considered immune to *Bacterium solanacearum*. These are listed in table 4. Five of the species found to be immune have been listed by various workers as susceptible. These are watermelon, *Crotalaria striata*, cotton, sweetpotato, and fireweed. References to literature citations in which reports of susceptibility are made are given in table 4. After receiving heavy stem inoculations

TABLE 4.—*Plant species immune to natural and artificial infection by Bacterium solanacearum*

Botanical name and report of susceptibility ¹	Common name	Approximate number of plants grown
Cultivated plants:		
<i>Citrullus vulgaris</i> Schrad. (23)	Watermelon	22
<i>Crotalaria intermedia</i> Kotschy		150
<i>Crotalaria incana</i> L.		150
<i>Crotalaria lanceolata</i> E. Mey.		150
<i>Crotalaria mazallaris</i> Klotzsch		150
<i>Crotalaria rutosa</i> L.		150
<i>Crotalaria spectabilis</i> Roth.		150
<i>Crotalaria striata</i> DC. (3)		150
<i>Crotalaria usaramensis</i> E. G. Baker		100
<i>Cucumis melo</i> L.	Cantaloup.	85
<i>Gossypium hirsutum</i> L. (18)	Cotton	84
<i>Hibiscus esculentus</i> L.	Okra	81
<i>Humulus lupulus</i> L.	Hops	12
<i>Ipomoea batatas</i> (L.) Lam. (13)	Sweetpotato	71
<i>Lespedeza striata</i> Hook. and Arn.	Japan clover	11
<i>Pisum sativum</i> var. <i>arvense</i> Poir.	Canadian field peas	2 5
<i>Sorghum vulgare</i> var. <i>saccharatum</i> (L.) Boerl.	Sorgo	58
<i>Trifolium incarnatum</i> L.	Crimson clover	2 5
<i>Vicia sativa</i> L.	Vetch	2 5
<i>Zea mays</i> L.	Corn	71
Wild plants:		
<i>Amaranthus spinosus</i> L.	Spiny pigweed	43
<i>Amaranthus retroflexus</i> L.	Pigweed	25
<i>Apocynum</i> sp.	Dogbane	4
<i>Bidens frondosa</i> L.	Sticktight	14
<i>Chenopodium album</i> L.	Lambsquarters	102
<i>Chenopodium botrys</i> L.	Jerusalem-oak	30
<i>Chrysanthemum leucanthemum</i> L.	Oxeye daisy	8
<i>Desmodium illinoense</i> Darl.	Beggarticks	6
<i>Desmodium ciliare</i> DC.	do.	8
<i>Diodia virginiana</i> L.	Larger buttonweed	8
<i>Eupatorium capillifolium</i> (Lam.) Small	Dogfennel	8
<i>Erechtites hieracifolia</i> (L.) Raf. (13)	Fireweed	108
<i>Euthamia minor</i> (Michx.) Greene	Flat-topped goldenrod	11
<i>Ipomoea hederacea</i> Jacq.	Morning-glory	12
<i>Ipomoea pandurata</i> (L.) Meyer	Wild potato	4
<i>Ipomoea purpurea</i> (L.) Lam.	Morning-glory	12
<i>Lonicera japonica</i> Thunb.	Japanese honeysuckle	6
<i>Lactuca sagittifolia</i> Ell.	Arrow-leaved lettuce	4
<i>Oenothera biennis</i> L.	Common oenothera	2
<i>Oxalis stricta</i> L.	Woodsorrel	10
<i>Passiflora incarnata</i> L.	Maypop	3
<i>Phytolacca americana</i> L.	Pokeweed	26
<i>Polygonum pennsylvanicum</i> L.	Smartweed	12
<i>Polygonum persicaria</i> L.	do.	36
<i>Portulaca oleracea</i> L.	Purslane	6
<i>Rubus</i> sp.	Blackberry	4
<i>Rumex acetosella</i> L.	Sheep sorrel	6
<i>Rumex obtusifolius</i> L.	do.	4
<i>Sida spinosa</i> L.	Ironweed	23
<i>Solidago canadensis</i> L.	Goldenrod	9
<i>Solidago nemoralis</i> Ait.	do.	4
<i>Solidago pinetorum</i> Small	do.	4
<i>Tecoma radicans</i> (L.) Juss.	Cow itch vine	8
<i>Trifolium arvense</i> L.	Rabbit-foot clover	13
<i>Verbascum thapsus</i> L.	Mullein	24
<i>Verbena urticifolia</i> L.	White vervain	15

¹ Reference is made by italic numbers in parentheses to literature citations (p. 439) in which reports of susceptibility were made.

² Inoculation only.

and after being grown on very highly infested soil, these species showed no indications of infection.

For certain of these species only a small number of plants were tested. However, extensive observations during the last four seasons on the weed floras of infested fields have been used to supplement the results obtained from the susceptibility tests.

DISCUSSION

The main interest of these experiments lies in the relation of the host range of *Bacterium solanacearum* to the control of Granville wilt by crop rotation. The results provide a basis for the experiments now in progress on the control value of various cultivated crops when used in rotations and on the effect of susceptible weeds on the degree of control obtained. Certain crop plants were not considered safe for use in rotations on infested land because they were included in the host list of *Bact. solanacearum*. Evidence of susceptibility lies in part in their susceptibility to inoculation. The results presented in table 2 and the illustrations in figures 4 and 5 show that some plants were positive to artificial stem inoculation and negative to natural infection.

If inoculation by artificial methods and subsequent reisolation of the parasite are accepted as adequate fulfillment of Koch's postulates, such plants must be considered as susceptible. However, the fact remains that they are not infected by the soil-borne organism when grown on very highly infested soil. It appears that when the organism is introduced through wounds into the stems of certain plants their mechanism of resistance is broken down. Such plants are susceptible as the result of artificial manipulation and hence are not susceptible to the disease in which primary invasion occurs through the roots from soil-borne infection. It is believed that these species should not be considered as host plants. The term "host plant" loses its practical significance if plants so listed do not develop the disease in the field and do not play an active part in the persistence and spread of the causal organism. This situation is similar to that found by Clayton (2), Johnson (11), Tisdale (21), and Young (24), who demonstrated that artificial methods might be misleading if exclusively used to determine host range of a number of parasites.

For the practical purposes of crop rotation, plants that are immune to natural infection should be safe for use on infested soil. Experiments by Garner, Wolf, and Moss (?) with cowpeas and experiments in progress by the author with cowpeas and soybeans show that rotations of these plants reduce the amount of wilt on succeeding crops of tobacco. Extensive farm experience corroborates these data.

SUMMARY AND RECOMMENDATIONS

The susceptibility of 90 plant species to *Bacterium solanacearum* E. F. Smith was established by (1) stem inoculation made with wedges of woody tissue from diseased tobacco plants, and (2) natural infection when grown in the field on naturally infested soil under very severe disease conditions. The species were divided into three classes on the basis of their reaction to these two tests: Class 1, susceptible to both stem inoculation and natural infection; class 2, susceptible to stem inoculation and immune to natural infection; class 3, immune under both methods of testing.

Class 1 includes 29 species. Of these tobacco, tomato, *Nicotiana rustica*, eggplant, jimsonweed, black nightshade, croton, Spanish-needles, 1 species of cocklebur, and nasturtium became 100-percent diseased.

Class 2 includes five species. Of these, cowpeas, soybeans, and velvetbeans were studied intensively because of their importance as field crops. A number of agronomic varieties of each were grown, and they remained immune under very severe disease conditions. All were susceptible after stem inoculation, but it was shown that the number of bacteria in the tissues of these plants was vastly smaller than the number present in infected tobacco. The organism was not able to multiply rapidly in the tissues of these plants. Since these species are immune to natural infection, it appears that they should not be listed as true hosts.

Class 3 includes 56 species that were immune to artificial inoculation and natural infection.

In conclusion, the following changes are recommended for the host list of *Bacterium solanacearum*:

(1) Remove from the host list: Sweetpotato, cotton, watermelon, fireweed, *Crotalaria striata*, velvetbeans, lima bean, soybeans, and cowpea.

(2) Add to the host list: *Xanthium pennsylvanicum* Wallr., *X. chinense* Mill., *Physalis pruinosa* L., *Aster pilosus* Willd., and *Ambrosia trifida* L.

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EFFECT OF TEMPERATURE ON SIZE OF EGGS FROM PULLETS IN DIFFERENT LATITUDES¹

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THE PROBLEM

It has been shown by Bennion and Warren (2)³ that high environmental temperatures materially influence the size of fowls' eggs. It is also well known that the eggs of pullets rapidly increase in size during the first few months of production. The few instances in which egg size has been recorded throughout the pullet year (2, 5) have shown the mean egg size to increase rapidly till early spring, after which it declined rather consistently throughout the summer. These records were taken in the central Temperate Zone.

The results of Bennion and Warren indicated that much of the reduction in egg size observed in late summer is associated with periods of excessively high temperature. In view of this fact it is of interest to compare the curves of annual egg size for pullets kept in different latitudes where the temperatures would vary greatly. It is not the purpose of this study to demonstrate the influence of temperature on egg size, since it is believed that this demonstration has been more convincingly made under controlled conditions (2). It is desired here to show how the curves of annual egg size may vary when observations are made under different temperature conditions.

MATERIALS AND METHODS

In order to secure data for the construction of annual egg-size curves for birds kept in various latitudes, records were obtained from scientific workers in different parts of continental United States, the Philippine Islands, Canada, and Scotland. The data used were secured from the records of agricultural colleges or from recognized egg-laying contests. Further information regarding the data is given in table 1.

In all instances daily egg-size records from individual birds were secured and from them mean daily egg size was calculated by the author. The minimum number of birds used for constructing a curve was 25 and the maximum 146. In all instances only the records of those pullets that laid fairly consistently over the entire period under consideration were used in the calculation, thus reducing the possibility of not having a uniform sample throughout the year. Various units of measure of egg size were found in the original data, but for all the curves constructed, the results were converted into weight in grams. Temperature was expressed in degrees Fahrenheit for the corresponding year. In some cases the original units of

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² The author is greatly indebted to the following workers for valuable data used in this study: C. G. Card, East Lansing, Mich.; F. A. Hays, Amherst, Mass.; V. S. Asmundson, Davis, Calif.; R. M. Sherwood, College Station, Tex.; N. R. Mehrhof, Gainesville, Fla.; W. A. Maw, Macdonald College, Quebec, Canada; B. F. Tinney, Charlottetown, Prince Edward Island, Canada; W. W. Bird, Nappan, Nova Scotia, Canada; F. M. Fronda, Los Banos, Laguna, P. I.; and the Department of Agriculture, Edinburgh, Scotland.

³ Italic numbers in parentheses refer to Literature Cited, p. 452.

measure of egg size were not so small as desired, but since the points on the curves were for means calculated from several records it is believed that the results are dependable. In all of the work any eggs that appeared to be yolkless or double yolked were eliminated from consideration. Since individual egg records were available, such abnormal eggs could usually be recognized. In the original summarization, curves were made for daily maximum temperatures and daily mean egg weights for each region, but to conserve space most of the curves presented were constructed from the mean of semimonthly periods calculated from the daily means. In each of the curves of egg size shown, horizontal lines are drawn at the 56-gm. weight and the 70° temperature for purposes of comparison. These two points were chosen because 56 gm. probably represents the ideal for egg weight and 70° F. seemed to be the minimum at which a pronounced effect was shown by egg size. It is the shape and not the level of the egg-weight curves that is of interest. The mean egg size for any group is probably an expression of inherent qualities that are not here considered.

TABLE 1.—*Sources of data used in this study*

Location	Degrees north latitude	Range of daily maximum temperature	Breed of chicken	Type of records	Year
		°F.			
Roslin, Scotland.....	56	32 to 76	{ White Wyandotte..... White Leghorn.....	{ Contest..	1935-36
Charlottetown, Prince Edward Island, Canada.....	46	{ -1 to 82 5 to 87	{ Barred Plymouth Rock.....	{ ..do.....	{ 1928-29 1930-31
Nappan, Nova Scotia, Canada.....	46	-7 to 84	{ White Leghorn..... Barred Plymouth Rock.....	{ ..do.....	1924-25
Macdonald College, Quebec, Canada.....	45	-4 to 88	{ White Leghorn.....	{ College..	1927-28
Amherst, Mass.....	42	12 to 96	{ Rhode Island Red.....	{ ..do.....	1935-36
East Lansing, Mich.....	43	3 to 106	{ White Leghorn..... Barred Plymouth Rock.....	{ Contest..	1934-35
Manhattan, Kans.....	39	{ 13 to 110 4 to 116	{ White Leghorn..... Crossbred.....	{ College..	{ 1921-22 1935-36
Davis, Calif.....	38	47 to 110	{ White Leghorn.....	{ ..do.....	1934-35
College Station, Tex.....	31	41 to 109	{ ..do.....	{ ..do.....	1933-34
Chipley, Fla.....	31	35 to 97	{ ..do.....	{ Contest..	1934-35
Los Banos, Laguna, P. I.....	14	77 to 96	{ ..do.....	{ College..	1934-35

MID-TEMPERATE ZONE DATA

The data collected in the mid-Temperate Zone should best show the effects of temperature on egg size since in this zone the greatest extremes in temperatures are found. Considerable data are available from Manhattan, Kans., one set of which is shown in figure 1. In this figure the relationship of the curves of daily maximum and of daily mean egg weight are shown for a group of 146 crossbred pullets mostly from the Black Minorca White \times Leghorn cross. It is seen that low winter temperature fluctuations have no influence on egg size, since the curve of egg size consistently rises until sometime in February. This rise is characteristic of pullets during the initial stages of egg production. After reaching the high point about the middle of February the egg-size curve begins to recede. From that time on, the curves of egg size and temperature are excellent counterparts of each other, the high points in the temperature curve being associated with points of small egg size. As will be shown in other

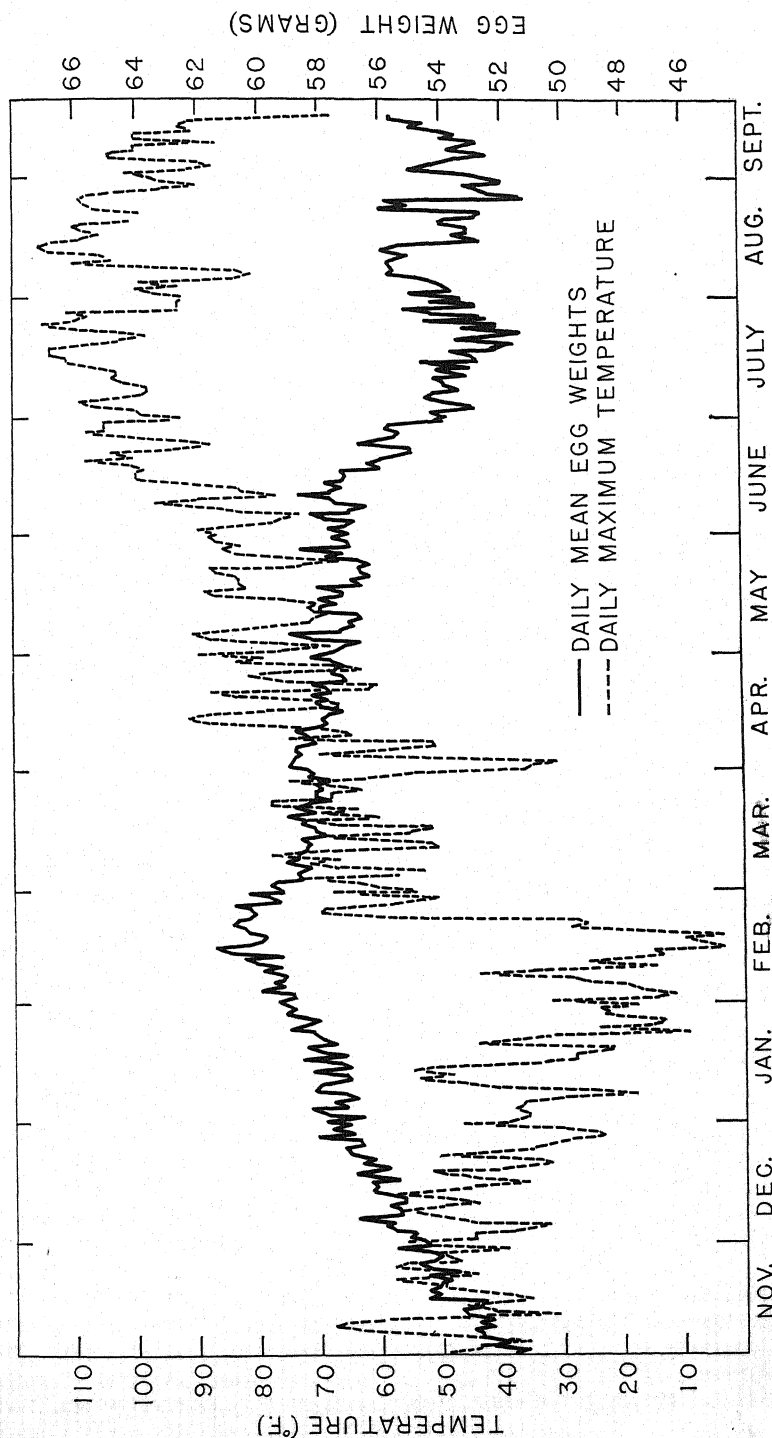


FIGURE 1.—Relation of curves of daily maximum air temperatures and daily mean egg weights for a group of 146 crossbred pullets kept at Manhattan, Kans., 1935-36.

graphs, when the maximum temperature consistently remains above 70° F. the egg size begins to decline, reflecting in inverse proportion the fluctuations in temperature. Any extreme changes in temperature extending over periods of a few days are reflected in fluctuations in egg size. From these results it would seem that much of the decline in egg size during the summer season at Manhattan, Kans., is due to the high temperatures of that section. This opinion is strongly confirmed by the results of Bennion and Warren (2) secured under controlled temperature conditions.

In the graphs showing egg size from various latitudes the data have been averaged for semimonthly periods. Although this tends to obscure the short period effects it shows to better advantage the annual trends. Figure 2, A, gives the data for a group of White Leghorns

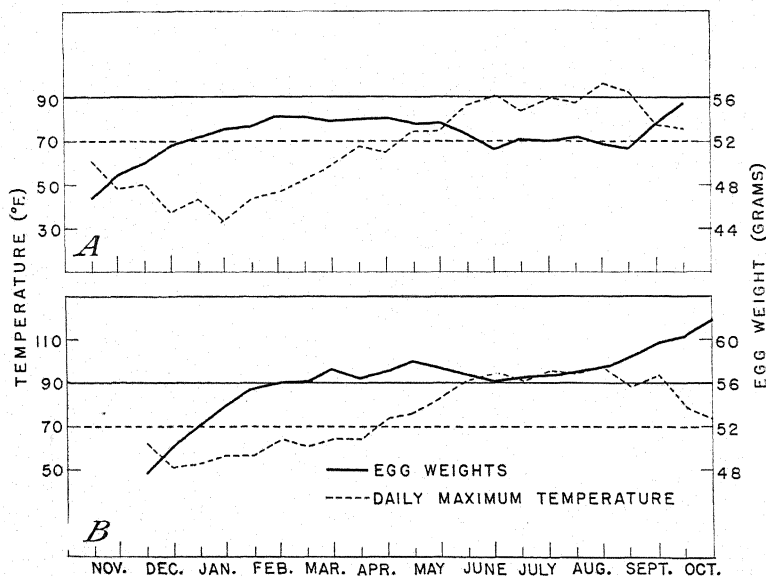


FIGURE 2.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in the mid-temperate zone, showing the influence of temperature on annual egg-size curves: A, 62 White Leghorns at Manhattan, Kans., 1921-22; B, 40 White Leghorns at Davis, Calif., 1934-35.

at Manhattan, Kans., for the year 1921-22. The trends are very similar to those of the crossbreds (fig. 1) except that a greater number of extremely high temperatures were encountered in 1935-36.

Figure 2, B, was constructed from data taken at Davis, Calif., which lies in approximately the same latitude as Manhattan, Kans. At Davis in 1935 a temperature above 70° F. was reached somewhat earlier than in the 1922 Kansas data. Figure 2 A, B, shows very similar summer maximum temperatures. The California egg-weight curve is similar to the Kansas one except that there is less depression of the curve in summer. A possible contributing factor not shown in the figures is the daily minimums. Those of California ranged from 10° to 18° lower than the minimums for Kansas. The cooler nights

may have afforded an opportunity for some recovery from the effects of the heat in California and so have accounted for less depression of the egg-weight curve. It is of interest to note the sharp increase of the California egg size in August and September following the rapid decline in temperatures at that season.

TROPICAL AND SUBTROPICAL DATA

Data were available for points in tropical and subtropical regions, including College Station, Tex.; Chipley, Fla.; and Los Banos, Laguna,

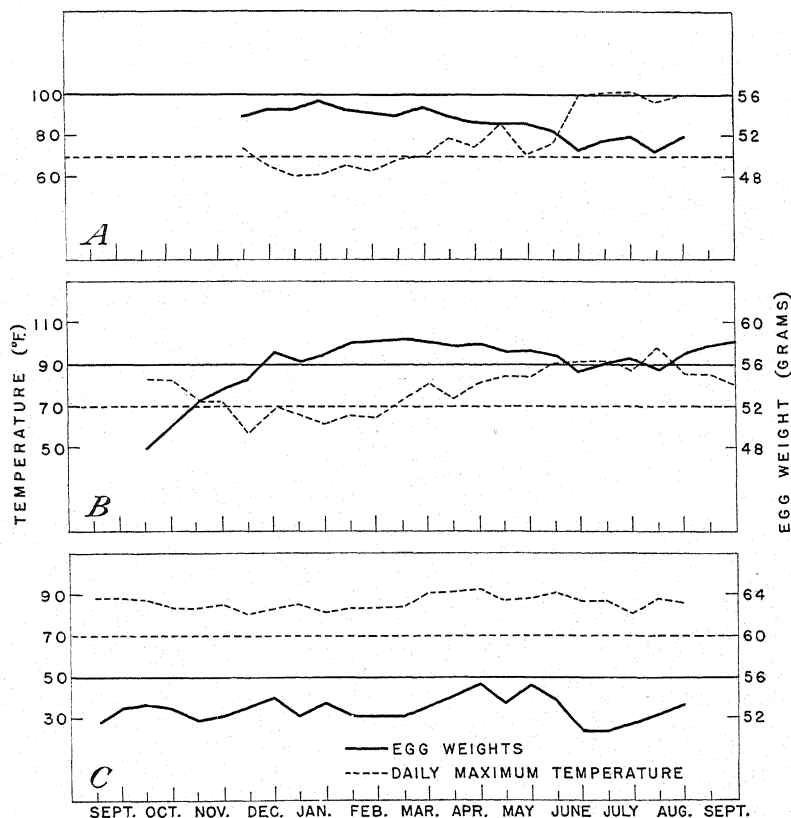


FIGURE 3.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in tropical and subtropical regions, showing the influence of temperature on annual egg-size curves: A, 45 White Leghorns at College Station, Tex., 1933-34; B, 51 White Leghorns at Chipley, Fla., 1934-35; C, 30 White Leghorns at Los Banos, Laguna, P. I., 1934-35.

P. I. (fig. 3). The curves from these regions are not strictly comparable with those from more northerly latitudes since the maximum temperatures for much of the winter season in the southern latitudes are near or above the 70° minimum at which temperatures are noticeably effective in reducing egg size. Thus the curve of egg size would tend to have its peak flattened, making the depression at the period of highest temperature appear less extreme. Such a condition is

evident in the Texas and Florida curves. The increase in egg size at the very end of the laying year, accompanied by a decline in temperature, is shown in the Florida data.

An interesting curve of annual egg size was secured from the data recorded at the agricultural college at Los Banos, Laguna, P. I. The location is near the Equator and the maximum temperatures are uniformly high throughout the year. From table 1 it will be seen that the extreme range of maximum temperature is 77° to 96° F. When averaged for 15-day periods as shown in figure 3, *C*, the temperature curve is almost a straight line at about 86°. The form of the curve of mean egg size differs considerably from those constructed from other data. Although there are fluctuations in egg size the trend approaches a horizontal line. Doubtless the uniformly high temperatures have suppressed the egg size throughout the entire year, thus preventing the attainment of a size that would have been possible under lower temperatures. There is an increase in egg size in April and May and a decline in June which have no accompanying variation in temperature. There was a marked increase in rainfall at about the time of the June decline, but it is not known whether this had a depressing effect.

NORTHERN LATITUDE DATA

In the more northerly latitudes data were available from East Lansing, Mich.; Amherst, Mass.; Macdonald College, Quebec, Canada; Charlottetown, Prince Edward Island, Canada; Nappan, Nova Scotia, Canada; and Roslin, Scotland. Conditions in these regions as they affect egg size are markedly different from those in the southern latitudes. In some of the northern localities the entire range of temperature is below the point at which egg size is noticeably affected by temperature, while in some of the southern localities from which data were obtained the entire temperature range was above this point.

In Massachusetts and Michigan (fig. 4) the extreme of high temperatures sometimes approached those reached in Kansas, but the periods of high temperature were less sustained and the daily minimum temperatures were considerably lower. The curves of egg size from Michigan and Massachusetts each showed a slight depression during July and August when the temperatures reached a maximum. However, the form of the curve is very different from that secured at Manhattan, Kans. The curve for East Lansing, Mich., follows nearly a straight line after reaching a maximum in February. The records from Michigan included data on both White Leghorns and Barred Plymouth Rocks, and it is to be noted that the curves for two breeds are almost identical.

The Canadian data tend to confirm the results from the northern United States, indicating that where the maximum temperature seldom exceeds 70° F. for periods of any length, the egg size is little depressed. Data were summarized from White Leghorns at Macdonald College, Quebec, and Barred Plymouth Rocks at Nappan, Nova Scotia, and Charlottetown, Prince Edward Island, there being results for 2 different years at Charlottetown. These data are presented in figures 5 and 6. Although there is some variation among the curves, the results are in close agreement in showing little or no

depression of egg size during the summer. The general form of the curves shows a gradual rise during the entire pullet year, although the slope is rather slight after February. The maximum temperature curve seldom exceeds 70° for the Canadian data and then remains so only briefly. These data indicate that where high temperatures do not interfere, there is an increase in egg size throughout the pullet year.

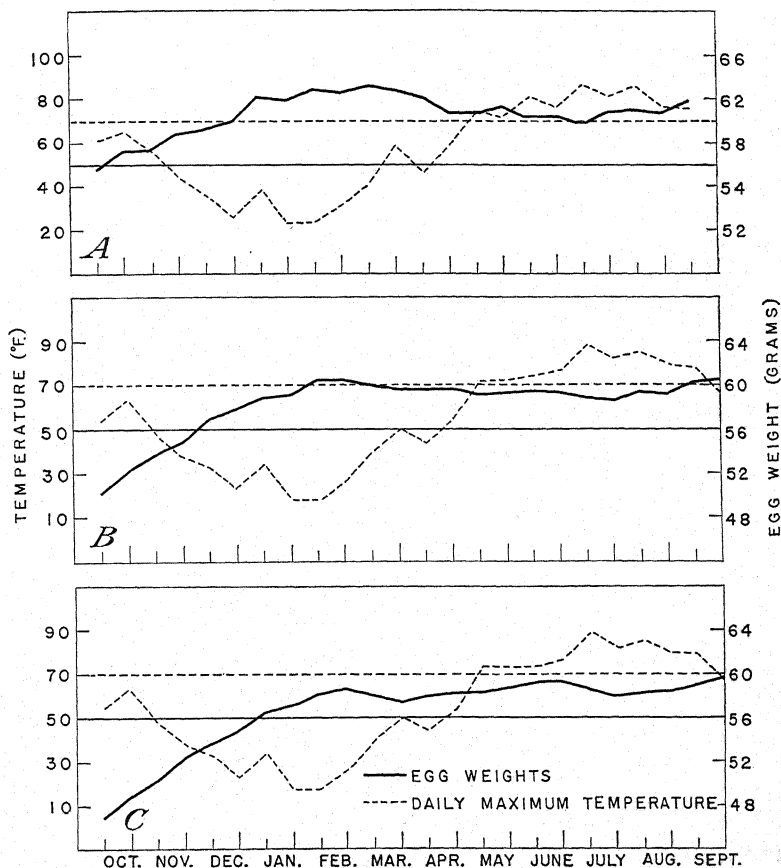


FIGURE 4.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in northern latitudes, showing the influence of temperature on annual egg-size curves: A, 25 Rhode Island Reds at Amherst, Mass., 1935-36; B, 106 White Leghorns at East Lansing, Mich., 1935-36; C, 61 Barred Plymouth Rocks at East Lansing, Mich., 1935-36.

Data were secured on White Wyandottes and White Leghorns at Roslin, Scotland. The curves in figure 7 for the two breeds during the same year are in close agreement. The mean maximum temperatures by 15-day periods never exceeded 70° F., although the daily maximum did reach 76° . The results here agree with those from the Canadian data in showing a maximum of egg size at the period of maximum temperatures during August and September. There was,

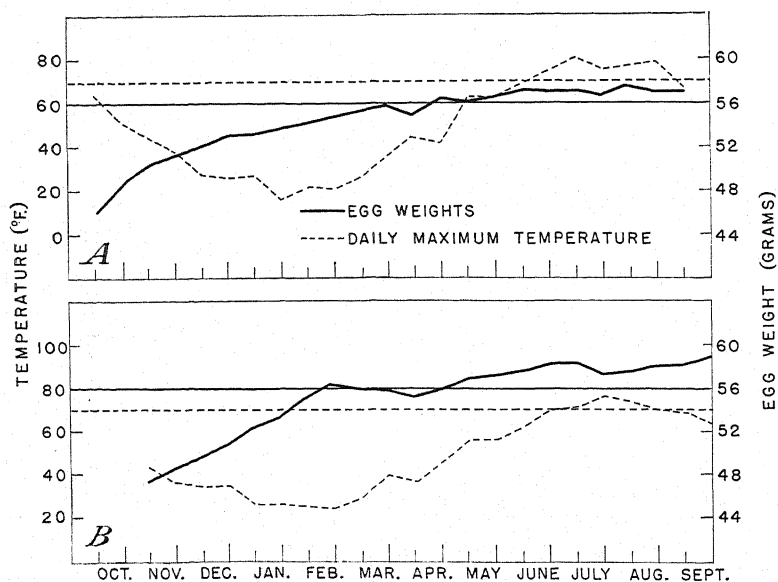


FIGURE 5.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in northern latitudes, showing the influence of temperature on annual egg-size curves: A, 83 White Leghorns at Macdonald College, Quebec, Canada, 1927-28; B, 76 Barred Plymouth Rocks at Charlottetown, Prince Edward Island, Canada, 1928-29.

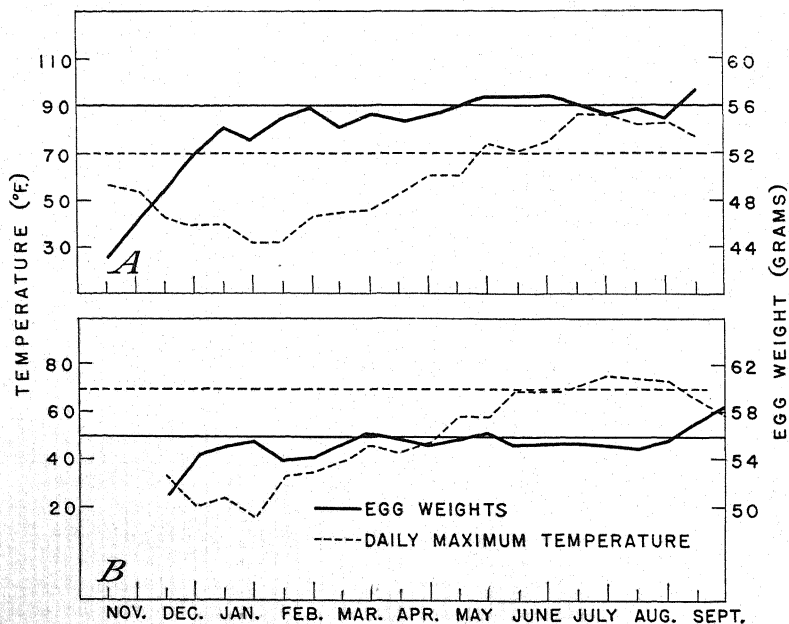


FIGURE 6.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets kept in northern latitudes, showing the influence of temperature on annual egg-size curves: A, 59 Barred Plymouth Rocks at Charlottetown, Prince Edward Island, Canada, 1930-31; B, 107 Barred Plymouth Rocks at Nappan, Nova Scotia, Canada, 1924-25.

however, an unexplainable decline in egg size during May and June when the temperature is not high enough to influence egg size. The results in this period are so far out of line with other data as to suggest the influence of some unidentified environmental factor.

DISCUSSION

From the data presented in this and earlier publications it seems fairly evident that egg size is materially reduced by high temperatures. It is recognized that the curve of annual egg size for any locality is representative of the effects of temperature for the particular year recorded and that the curve might vary considerably under a different set of conditions. The use of maximum temperatures rather than the mean daily temperatures might be questioned, since the relief which the bird gets at night is probably a contributing factor

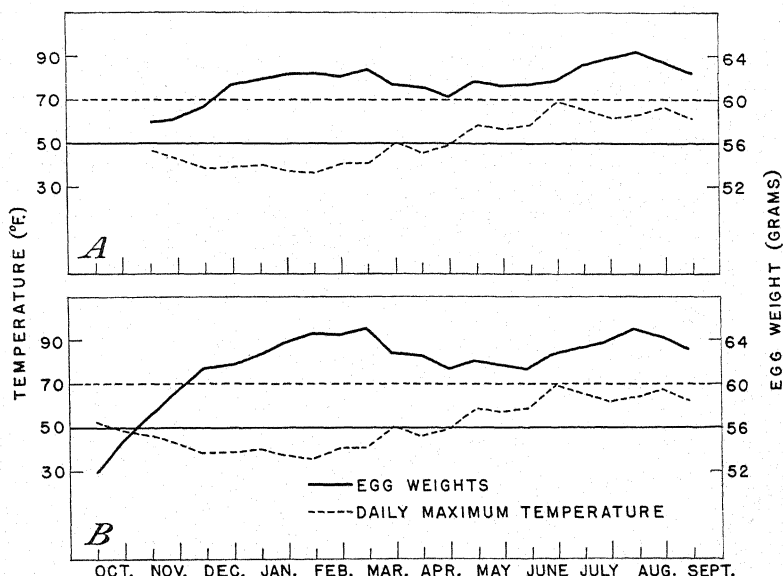


FIGURE 7.—Mean maximum air temperatures and mean egg weights at semi-monthly periods for groups of pullets at Roslin, Scotland, 1935-36, showing the influence of temperature on annual egg-size curves: A, 42 White Wyandottes; B, 41 White Leghorns.

in the reaction of her egg size to temperatures. However, the extreme height to which the day temperatures go is also an important factor which would be somewhat masked by the daily mean. Where data were available for more than one breed for the same year and locality, the curves were very similar, indicating that the available samples were representative and that many of the minor fluctuations in egg size are the result of unidentified environmental influences. Low winter temperatures seem to have no influence on egg size.

No explanation is offered as to the manner in which high temperatures produce their effect. Galpin (4) attempts to explain seasonal changes in egg size by attributing them to variations in the metabolic

level of the hen. He finds a parallelism between seasonal curves of thyroid weight and egg weight. Riddle (?) found a similar seasonal variation in thyroid weight in pigeons which he believed to be a response to external temperature variations. Asmundson and Pinsky (1) found that the feeding of thyroxin decreased egg size. These results as a whole suggest a relationship between egg size and the functioning of the thyroid which in turn may be conditioned by external temperature variations. The problem of how the three factors are related requires further investigation. The study of Bennion and Warren (2) showed that all parts of the eggs were reduced in weight but that the percentage decrease was greatest in the shell and egg white. This would indicate that the functioning of the oviduct is more affected than that of the ovary.

From the data here presented it is difficult to state the exact minimum at which temperature becomes a factor in influencing egg size. These data are from the pullet year and there is evidence of an inherent

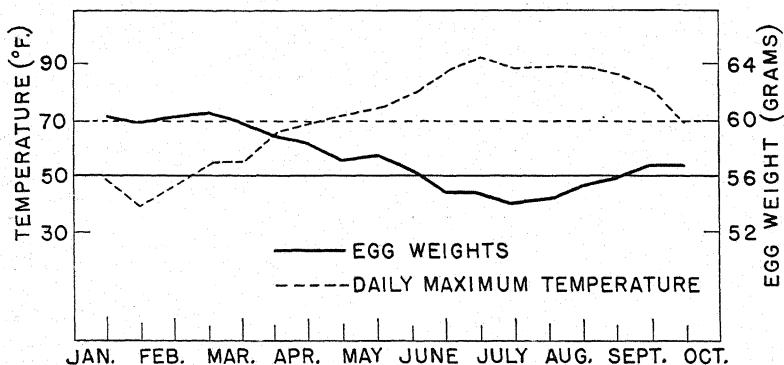


FIGURE 8.—Mean maximum air temperatures and mean egg weights at semi-monthly intervals for 20 White Leghorn hens (second-year) at Manhattan, Kans., showing the influence of temperature on annual egg-size curves, 1923-24.

tendency of eggs to increase in size during the year. If pullets encounter temperatures high enough to depress egg size, the antagonistic influences will neutralize one another and the minimum point at which temperatures are effective will be obscured. Only when the temperatures are high enough to counteract the tendency for eggs to become larger as the year progresses, will the influence of high temperature be evident. For this reason data on pullets are not very crucial in determining the minimum at which temperatures are effective in depressing egg size. Results from hens in their second or later laying years would be more exact for this purpose. In figure 8 the data from hens do indicate that egg size is depressed by temperatures as low as 50° F. Most of the curves involving pullet data do not show much effect of temperature at this low point. In many of the curves presented in this paper it is evident that when the maximum temperature exceeds 70° for very many days there is a depressing effect on the egg size. There are other instances in which it appears that sudden increases in temperature at 40° and 50° may temporarily suppress the size of the egg. Precipitous changes in temperature, within the effective range, seemed to have a more pronounced influence on egg

size. Lorenz and Almquist (6) found evidence of a decrease in egg weight proportional to the increase in temperature between 40° and 90°. Bruckner (3) found that under controlled conditions in a house where the temperature was held at a mean of 50° there was evidence of a depressing effect on egg size in winter as compared with the egg size of birds subjected to the normal temperatures of New York.

The fact that egg size is so sensitive to changes in environmental temperatures has a significant bearing on the problem of what constitutes an accurate measure of egg size. If records for short periods are to be utilized, the temperature encountered during those periods must be taken into consideration. Even if the mean egg size for the entire pullet year is used as a measure, the worker is confronted with the temperature problem in comparing one year's results with another. This problem is also encountered in selection studies in comparing dam and daughter egg size, and it is a factor that cannot be ignored in genetic studies where succeeding generations must be compared.

The question may be raised as to what constitutes a normal curve of egg size for the pullet year. An examination of results secured in the more northern latitudes indicates that if high temperatures do not interfere, egg size continues to increase throughout the entire first year of production. If this be true then pullets kept in the more southern latitudes never reach their maximum potential egg size. This has a very definite bearing upon any Nation-wide breeding program in which birds from different regions are compared for egg size.

The fact that egg size usually declines during the summer in many regions is a matter of common knowledge, and the opinion has sometimes been held that the decline is the expression of the fatiguing influence of a long period of production. The lack of any decline in egg size near the end of the laying year in the data from northern latitudes, however, does not support this view. Even more convincing refutation is seen in the curves which show a depressing effect of summer temperatures. In such instances, there was usually a rapid increase in egg size at the very end of the pullet year, provided the temperature decreased significantly. If fatigue were a factor in producing small egg size in summer then the recovery at the very end of the laying year would not be expected.

In order to determine whether the depressing effects of high temperature on egg weight may be found in the eggs of hens as well as pullets, data were summarized from the second-year records of a group of White Leghorns. Although the data are rather limited the form of the curve (fig. 8) is quite similar to that of pullets from the same latitude, except that egg size for the older birds started at a point near the maximum for the pullet year. There was the same striking summer depression in egg size that characterized the data from pullets.

White Leghorns, Barred Plymouth Rocks, Rhode Island Reds, White Wyandottes, and crossbreds were included among the breeds investigated, and it would seem, therefore, that the results secured would apply generally.

There were a few instances of depression of egg size with which change in temperature could not be associated. This would indicate that factors of the environment other than temperature may influence egg size. It is improbable that temperature, although important, is the only environmental factor that conditions egg size. It is also

possible that some instances of unaccounted-for variation in egg weight might have been due to the fact that the weighing devices were temporarily out of adjustment.

Although the data here presented are all in the form of means, several instances were observed in which individuals appeared to vary in their susceptibility to the depressing influence of high temperatures. This would suggest the possibility that birds might be bred for ability to continue with satisfactory egg size when subjected to adverse temperature conditions.

SUMMARY

Data were secured on egg size from 11 localities in latitudes extending from the Equator to as far north as Scotland. Curves showing mean daily egg weights together with the daily maximum temperatures for each locality were drawn for the pullet laying year. A comparison of the various curves of annual egg size showed that temperature was a very important factor in determining the shape of the curve. In most instances egg size increased rapidly for the first few months as a result of approaching physiological maturity. Following this, egg size was found to bear a close relationship to the temperatures prevailing. Excessively low temperatures seemed to have no effect on egg size, but after the daily maximum temperatures exceeded 70° F. for a period of a few days or more, egg-size fluctuations usually showed a close correlation with those of temperature. Where the maximum temperature seldom exceeded 70° egg size increased throughout the entire pullet year, with the rate of increase relatively slow in the last half of the year. In localities where summer temperatures were high, the summer egg size was greatly depressed, and the bird was prevented from expressing its maximum potentialities for egg size unless the temperature dropped below 70° at the end of the laying year. If this decline in temperature occurred, the egg size increased rapidly thus indicating that the summer decline was not the result of any fatiguing effect of a long period of production.

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VIABILITY OF POLLEN AND OVULES OF BARLEY AFTER COLD STORAGE¹

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INTRODUCTION

The pollen of many plants can be kept viable during shipment or storage for the pollination of later maturing varieties. However, no very satisfactory method has been found for storing pollen of barley (*Hordeum* spp.), because of its great sensitivity to variations in relative humidity. The pistil of barley, on the other hand, is much less easily injured.

This study of the effect of various periods of cold storage upon the reproductive processes of the barley plant was made to determine (1) seed setting in unemasculated spikes allowed to resume activity after cold storage, (2) the viability of pollen stored in unemasculated spikes, and (3) the seed productivity of ovules in stored emasculated spikes. A set of seed involves not only the retention of viability in the pollen and ovules but also the dehiscence of anthers and accompanying pollination of stigmas after storage.

MATERIALS AND METHODS

The barley varieties utilized in this study (table 1) were grown during the winter and spring of 1937-38 at the Arlington Experiment Farm, Arlington, Va., in the greenhouse or in field plots.

TABLE 1.—*Barley varieties grown at Arlington, Va., during winter and spring of 1937-38*

Variety	C. I. ¹ No.	Description
Hannchen.....	531	2-rowed awned, spring.
Spartan.....	5027	Do.
Wisconsin Pedigree 38.....	5105	6-rowed awned, spring.
Smooth Awn 86.....	6268	6-rowed awned, winter.
Beardless 6.....	2746	6-rowed hooded, winter.
Hooded 6.....	6270	Do.
Brugh 76.....	6477	Do.

¹ Accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

Spikes at stages before anther dehiscence were prepared for emasculation by clipping off the distal portion of the boot leaf just below the spike, removing the lateral florets, and cutting off the lemmas and palets of the central florets just above the distal tips of the anthers. They were then emasculated and bagged as for hybridization, except that a slightly greater range of maturity was included. One to five days later, when the flowers exhibited a rather wide range of receptivity as judged by the spread of the lemma and palelet and extrusion

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of the stigma, the culms bearing these emasculated spikes were clipped off above the third node below the spike, so as to include the remnant of the boot and the entire leaf just below it. The cut ends were then placed in flasks containing tap water, the spikes being protected from excessive dehydration by glassine bags, and put into dark cold storage. With each lot of emasculated spikes were stored culms bearing unemasculated spikes with undehisced anthers, as a source of stored pollen.

The spikes from greenhouse-grown Hannchen barley were held in refrigerated chambers at 36° and 40° F., while those from the remaining varieties grown in field plots were stored at 36°, 40°, and 50°. All three chambers were dark and were maintained at a relative humidity of about 82 percent. Fresh pollen and ovules from later plants of the same variety were available throughout most of the experiments; but for testing the viability of stored pollen and ovules in the field-grown material the supply was supplemented subsequently with spikes from later maturing plots on account of the rapid exhaustion of viable pollen and productive ovules. Toward the end of the experiment fresh pollen from the two spring-sown varieties, Spartan and Wisconsin Pedigree 38, was used.

To test the ability of unemasculated stored spikes to set seed, a few were selected at random and placed in Erlenmeyer flasks containing tap water.

For testing stored pollen in the greenhouse experiment, fresh unpollinated spikes were always available, and flowers on these were dusted with stored pollen and allowed to mature seed upon the plant. For testing stored field pollen it was necessary to use fresh or stored spikes that had been cut from the plant. Since much of the stored pollen did not dehisce readily, the procedure of dusting the same flower with pollen from several spikes was followed. The spikes dusted with stored pollen were then placed in tap water and allowed to develop.

In testing the productivity of ovules of stored emasculated spikes, a number of spikes were chosen that exhibited as wide a range of flower receptivity as seemed desirable. These were pollinated with fresh pollen, when available, or with the pollen from the unemasculated spikes held in cold storage. The pollinated spikes were protected with glassine bags and likewise placed in flasks containing tap water. All the flasks containing the excised greenhouse spikes were then set on a well-lighted greenhouse bench, and those containing the field-plot spikes were staked upright in a bed in a nursery cage outside and allowed to resume activity. When the culms had largely lost their chlorophyll and seed development had apparently ceased, the spikes were removed to the laboratory, placed in paper envelopes, and dried.

EXPERIMENTAL RESULTS

SEED PRODUCTION OF STORED UNEMASCULATED SPIKES

Table 2 gives the seed set on the unemasculated spikes of greenhouse-grown plants held in tap water under favorable growing conditions after four periods of storage. Few seeds were set, but no spike failed entirely except the two stored for 21 days at 40° F.

In the field-grown barley (table 3), 29 days of storage at 36° permitted the development of 7 seeds in 1 out of 8 spikes. One seed developed in 1 out of 9 spikes that had been kept at a temperature of

40° for 26 days, and 10 seeds were found in 2 of the 12 spikes that had been stored 14 days at 50°. Photographs of the field-grown seeds are shown in figure 1.

TABLE 2.—Seed set on unemasculated spikes of greenhouse-grown barley, grown in tap water after storage at 36° and 40° F.

Storage period (days)	36° F.		40° F.		Storage period (days)	36° F.		40° F.	
	Spikes	Seed set	Spikes	Seed set		Spikes	Seed set	Spikes	Seed set
	Number	Number	Number	Number		Number	Number	Number	Number
9.....	5	15	5	7	16.....	3	11	3	9
13.....	5	14	5	9	21.....	3	3	2	0

TABLE 3.—Seed set on unemasculated spikes of field-grown barley, grown in tap water after storage at 36°, 40°, and 50° F.

Storage period (days)	36° F.			40° F.			50° F.		
	Spikes		Seeds	Spikes		Seeds	Spikes		Seeds
	Total	Fertile		Total	Fertile		Total	Fertile	
	Number	Number	Number ¹	Number	Number	Number	Number	Number	Number
14.....	11	11	(¹)	12	5	22	12	2	10
19.....	9	2	5	8	0	0	7	0	0
26.....	9	0	0	9	1	1
29.....	8	1	7	6	0	0
31.....
35.....	5	0	0	7	0	0
39.....	12	0	0	12	0	0
42.....

¹ Many seeds.

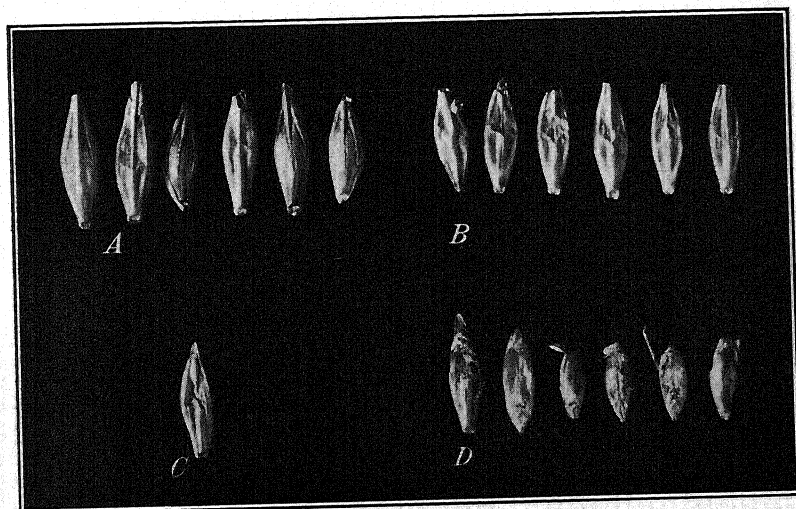


FIGURE 1.—A, Field-grown Beardless 6 barley matured on the plant. (Germination test in row 10, fig. 3.) B, Seed from unemasculated spikes stored 29 days at 36° F. (Germination test in row 11, fig. 3.) C, Seed from unemasculated spike stored 26 days at 40°. (Germination test in row 12, fig. 3.) D, Seed from unemasculated spike stored 14 days at 50°, badly moth-eaten. (One seed produced the plant shown in row 13, fig. 3.)

Unemasculated spikes from the greenhouse gave some indications of growth during storage. Two days after the culms were put into the chambers, the awn tips had extruded from the boot about 10 mm., and after 3 weeks the maximum growth measured was about 15 mm. Normal extrusion of the awn of Hannchen in the greenhouse was 23.5 mm. in 24 hours.² The flowers opened during storage if they were sufficiently mature when placed in storage, but only an occasional spike showed elongation of the anther filament. Only a small proportion of the spikes were harvested at the proper stage to produce dry pollen in ripe anthers immediately after removal from cold storage.

VIABILITY OF POLLEN IN REFRIGERATED BARLEY SPIKES

The viability of pollen from the spikes grown in the greenhouse was tested, after five periods of storage in the spike, on receptive flowers of growing greenhouse plants. In table 4 it is seen that pollen stored for 21 days at 36° F. was still able to produce seed, while that stored for the same length of time at 40° failed entirely. Nine days of storage gave a very poor seed set at 36° and none at 40°. This pollen, when germinated on receptive flowers and examined, showed a viability of 57 percent for storage at 36° and but 29 percent for storage at 40°, with many of the pollen tubes growing weakly and abnormally.

TABLE 4.—Seed set by pollen from spikes of greenhouse-grown barley, stored at 36° and 40° F., upon flowers of receptive greenhouse plants

Storage period of pollen (days)	36° F.				40° F.			
	Spikes	Flowers	Seeds	Seed set	Spikes	Flowers	Seeds	Seed set
	Number	Number	Number	Percent	Number	Number	Number	Percent
6.....	1	34	16	66.7	2	42	3	7.1
9.....	1	19	1	5.3	1	21	0	0
13.....	3	49	48	98.0	3	48	44	91.7
16.....	3	27	19	70.4	3	42	4	9.5
21.....	3	26	5	19.2	3	18	0	0

For testing the pollen of refrigerated field-grown culms it was necessary in most cases to use emasculated spikes that also had been stored. The period of storage in each case is indicated in table 5. At 36° F., pollen stored 26 days produced one seed in a spike that had been stored 15 days and three seeds in a spike stored for 26 days (fig. 2, A). This pollen was noted as "good." After this date the pollen deteriorated rapidly, and ovules stored longer than 26 days were very poor as pollen testers.

At 40° F., 1 spike stored 19 days had anthers dehiscing sticky pollen, which produced 4 seeds on 20 flowers stored 19 days at 36° (fig. 2, B). Pollen spikes stored 10 days at 50° were flowering, but the anthers were still striped with green, while on the fourteenth day the flowers were generally open, with anthers extruded and pollen scarce. No seeds set on 18 flowers. Dry pollen was noted on the nineteenth day, but no seeds were produced.

A good seed set resulted from pollen and ovules, both stored 19 days at 36° F. The longest successful pollen storage at 36° was 26 days, and at 40° it was 19 days. No seed was obtained from pollen stored at 50°.

² Unpublished 1920 data.

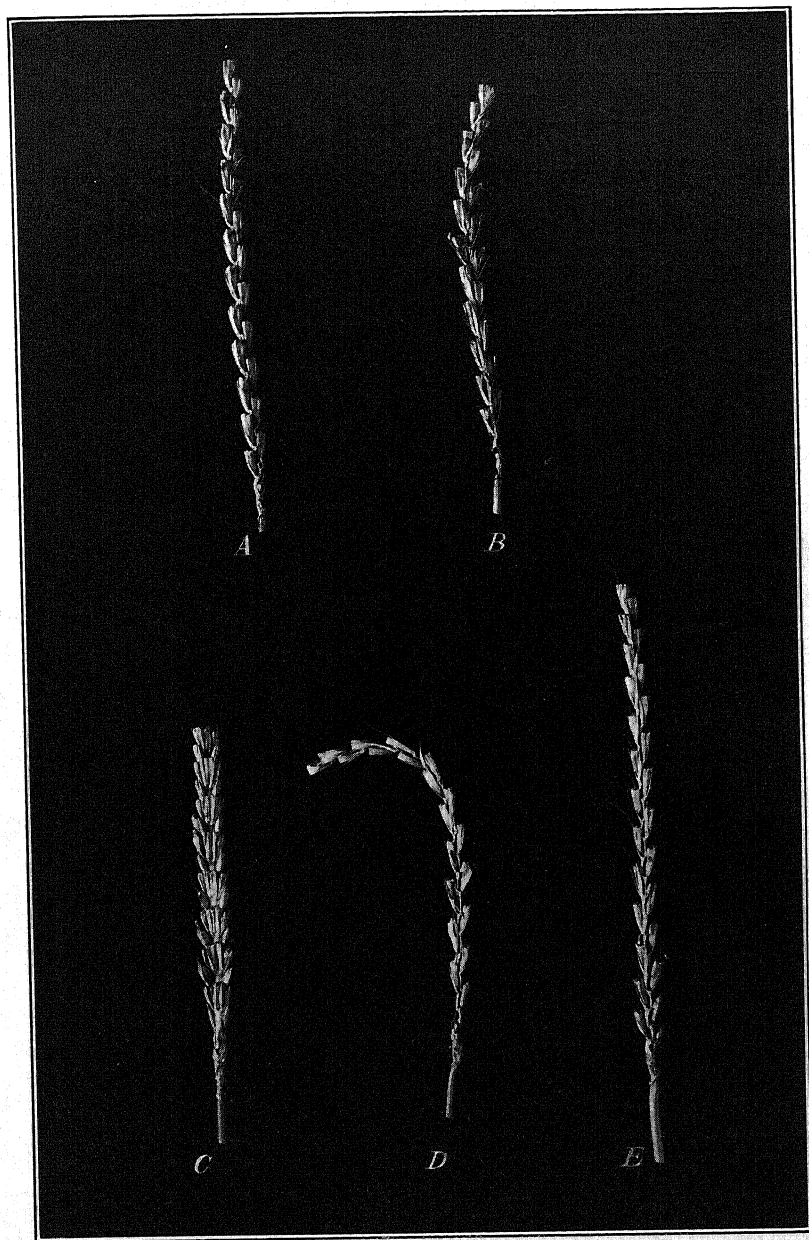


FIGURE 2.—A, Spike showing 3 seeds produced by pollen stored 26 days at 36°. F, upon ovules stored 26 days at 36°. (Germination test in row 14, fig. 3.) B, Spike showing 4 seeds produced by pollen stored 19 days at 40° upon ovules stored 19 days at 36°. (Germination test in row 15, fig. 3.) C, Emasculated spike stored 42 days at 36°, which set 5 lateral seeds after being dusted with fresh pollen. (Germination test in row 16, fig. 3.) D, Emasculated spike stored 29 days at 40°, which set 1 seed after being dusted with pollen stored 18 days at 36°. (Did not germinate.) E, Emasculated spike stored 14 days at 50°, which set 1 seed after being dusted with fresh pollen. (Germination test in row 17, fig. 3.)

TABLE 5.—Seed set by pollen from spikes of field-grown barley stored at 36°, 40°, and 50° F., upon best available ovules

Storage period of pollen (days)	36° F.						40° F.					50° F. 1 pollination 2	
	Pollination			Seed set			Pollination 2		Seed set			Spikes	Flowers
	Spikes	Ovules stored	Flowers	Spikes fertile	Seeds	Percent	Spikes	Flowers	Spikes fertile	Seeds	Percent		
	No.	Days	No.	No.	No.		No.	No.	No.	No.		No.	No.
8.....	2	19	36	2	28	77.8							
14.....	1	0	20	1	7	35.0	1	21	1	6	28.6	1	18
15.....	3	26	48	3	11	22.9							
16.....	1	35	18	0	0	0							
18.....	3	29	41	1	1	2.4							
19.....	2	19	42	2	17	40.5	1	20	1	4	20.0	1	17
20.....	1	39	18	0	0	0							
24.....	3	35	56	0	0	0							
26.....	1	1	15	1	1	9.5	1	15	0	0	0		
26.....		26	23	1	3								
29.....		2	29	36	0		1	23	0	0	0		
35.....	1	35	19	0	0	0	1	17	0	0	0		
39.....							1	19	0	0	0		

1 No seed set at 50° F.

2 Ovules same age as pollen but stored at 36° F.

SEED PRODUCTION AFTER COLD STORAGE OF EMASCULATED SPIKES

As would be expected, growth and development in emasculated spikes were greatly slowed down in cold storage, the normal cycle of flower opening and gradual closing proceeding in a much more leisurely manner. During the later storage stages the stigma hairs appeared withered at the tips but looked normal throughout most of their length.

Fifty spikes of greenhouse-grown Hannchen barley, emasculated during the period from March 9 to 13, were cut off and stored on March 14, 25 being stored at 36°, and 25 at 40° F. Spikes were moved at intervals from the refrigerator to the greenhouse, where they were dusted with fresh pollen and left with cut ends in tap water, on a well-lighted bench. Table 6 shows the percentage of seed set in emasculated spikes stored 7 to 21 days before pollination.

TABLE 6.—Seed set on emasculated spikes of greenhouse-grown barley when pollinated with fresh greenhouse pollen after cold storage

Storage period (days)	36° F.					40° F.				
	Pollination		Seed set			Pollination		Seed set		
	Spikes	Flowers	Spikes fertile	Seeds	Percent	Spikes	Flowers	Spikes fertile	Seeds	Percent
	Number	Number	Number	Number		Number	Number	Number	Number	
7.....	4	81	3	29	35.8	5	107	1	1	0.9
10.....	5	108	5	83	76.8	5	114	4	66	57.9
14.....	5	111	4	38	34.2	5	114	5	56	49.1
17.....	5	124	3	28	22.6	5	110	3	24	21.8
21.....	6	133	2	11	8.3	5	114	2	15	13.2

An examination of the dates of emasculation of the 50 greenhouse grown spikes showed that by far the best seed set was obtained when refrigeration was begun 3 days or less after emasculation. Consequently, late in April, 125 spikes of Beardless 6 barley were emasculated during a 3-day period, divided into three lots, and stored at 36°, 40°, and 50° F. Unemasculated spikes as a source of pollen were stored with each lot from the following varieties: Beardless 6, at the same time; Smooth Awn 86 and Brugh 76, 11 days later; and Hooded 6, 19 days later. Nursery-grown Spartan and Wisconsin Pedigree 38 supplied the fresh pollen used toward the end of the experiment. Table 7 shows the seed set upon stored spikes dusted with the best pollen available.

TABLE 7.—Seed set in emasculated spikes of field-grown barley after cold storage at 36°, 40°, and 50° F., by pollen fresh or stored at 36° F.

Storage period of ovules (days)	36° F.						40° F.						50° F.					
	Pollination			Seed set			Pollination			Seed set			Pollination			Seed set		
	Spikes	Pollen stored	Flowers	Spikes fertile	Seeds	Percent	Spikes	Pollen stored	Flowers	Spikes fertile	Seeds	Percent	Spikes	Pollen stored	Flowers	Spikes fertile	Seeds	Percent
	No.	Days	No.	No.	No.		No.	Days	No.	No.	No.		No.	Days	No.	No.	No.	
8	1	19	21	1	12	57.1	—	—	—	—	—	—	—	—	—	—	—	—
10	1	29	14	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—
14	2	0	37	2	25	67.6	5	3	98	5	50	51.0	5	3	98	1	1	1.1
15	1	26	19	1	1	5.3	—	—	—	—	—	—	—	—	—	—	—	—
19	2	8	36	2	28	77.8	4	8	66	1	3	4.5	8	8	156	0	0	0
26	3	15	48	3	11	22.9	5	15	105	2	3	2.9	—	—	—	—	—	—
29	3	18	41	1	1	2.4	5	18	80	1	1	1.3	—	—	—	—	—	—
31	5	0	91	4	19	20.9	4	0	78	0	0	0	—	—	—	—	—	—
35	1	16	18	0	0	0	4	16	70	0	0	0	—	—	—	—	—	—
39	2	0	29	0	0	0	5	0	82	0	0	0	—	—	—	—	—	—
42	4	0	109	1	5	4.6	—	—	—	—	—	—	—	—	—	—	—	—

Under storage at 36° F. the flowers of emasculated spikes opened and finally closed, seemingly in a perfectly normal manner, but at a much slower rate than under field conditions. At 40° flower opening was much more rapid and all flowers were closed on the thirty-ninth day of storage, while at 50° the flowers had opened and nearly all were closed by the fourteenth day of storage.

The foliage color of the culms stored at 36° F. was well preserved after 42 days of storage, but at 40° the leaves had begun to yellow after 19 days, and at 50° both spikes and leaves were quite yellow on the nineteenth day and chlorotic by the twenty-sixth day.

Fresh pollen was used on the ovules stored 14, 31, 39, and 42 days, with a generally decreasing seed set, as would be expected. Likewise, the productiveness of ovules stored at 36° F. varied roughly with the freshness of the pollen used. Fresh pollen on the fourteenth day gave a higher percentage of seed set than 19-day pollen on the ovules stored 8 days; the 10-day ovules dusted with pollen stored 29 days yielded nothing, while the 15-day ovules gave a 5.3 percent seed set with pollen stored 26 days. An exceptionally high seed set of 77.8 percent was obtained with ovules stored 19 days and dusted with 8-day pollen. A considerable number of flowers seemed quite unaffected by excision and storage for nearly a month. On the twenty-sixth day the seed

set of the spikes stored at 36° was 22.9 percent, but it dropped to 2.4 percent on the twenty-ninth day. However, with fresh pollen on ovules stored 31 days, a set of 20.9 percent was obtained. No further seed was obtained until the forty-second day, when 1 spike of the 4 that remained in storage had 5 open flowers and set 5 lateral seeds (fig. 2, *C*). The remaining 3 spikes had no open flowers. They presumably had opened and closed. It is worthy of note that the fertile spike mentioned was the only one of the 125 that had had all 6 rows of flowers emasculated.

The productivity of ovules stored at 40° F. decreased with storage age. Thus, ovules stored 29 days produced one seed (fig. 2, *D*), which did not grow. Fresh pollen was not available until the thirty-first day of storage, when, for the first time, no seed was produced.

Storage at 50° F. produced rapid deterioration of the ovule, since but 1 seed set on the fourteenth day out of 93 flowers dusted with pollen stored 3 days (fig. 2, *E*).

Germination tests of seed obtained in both greenhouse and field plot experiments were made on filter paper in Petri dishes at 15° C. for 3 days. Representative seedlings were then transplanted to a greenhouse flat. Table 8 shows the results of these tests, and figure 3 shows the seedlings as they appeared 9 days after transplanting. After 8 weeks of growth in the flat all plants shown in figure 3 except one in row 9 were still living and in spite of the crowding seemed normal. Plants in rows 10, 11, 12, 13, 15, and 16 were shooting or fully headed. Seeds from spikes emasculated or stored or both suffered a marked reduction in weight, but a seemingly normal plant was produced from a seed weighing but 4.6 mg. (row 14, plant 1), as compared with a weight of 35.7 mg. for seeds produced on a normal plant.

TABLE 8.—*Germination of seeds from normal and stored spikes of barley*

GREENHOUSE-GROWN HANNCHEN

Seed- ling row No. (fig. 3)	Treatment	Storage		Average seed weight	Seeds	Seeds germi- nated
		Duration	Temper- ature			
		Days	° F.	Milligrams	Number	Number
1.....	Matured on plant.....			49.5	13	13
2.....	Emasculated, pollinated, and matured on plant.....			22.9	38	38
3.....	Emasculated, pollinated, excised, and grown in tap water.....			12.9	59	59
4.....	Unemasculated, excised, and grown in tap water.....	21	36	8.8	3	1
5.....	do.....	16	40	16.7	9	9
6.....	Stored pollen on excised emasculated spikes.....	21	36	19.3	5	5
7.....	do.....	16	40	18.3	4	4
8.....	Emasculated spikes stored in tap water and pollinated.....	21	36	8.8	11	10
9.....	do.....	21	40	7.9	15	14

FIELD-GROWN BEARDLESS 6

10.....	Matured on plant.....			35.7	6	5
11.....	Unemasculated, excised, and grown in tap water.....	29	36	23.8	7	6
12.....	do.....	26	40	19.2	1	1
13.....	do.....	14	50	12.4+	10	11
14.....	Stored pollen on excised emasculated spikes.....	26	36	5.1	3	2
15.....	do.....	19	40	7.5	4	1
16.....	Emasculated spikes stored in tap water and pollinated.....	42	36	13.2	5	4
.....	do.....	29	40	2.0	1	0
17.....	do.....	14	50	13.1	1	1

+ Sample badly moth-eaten.

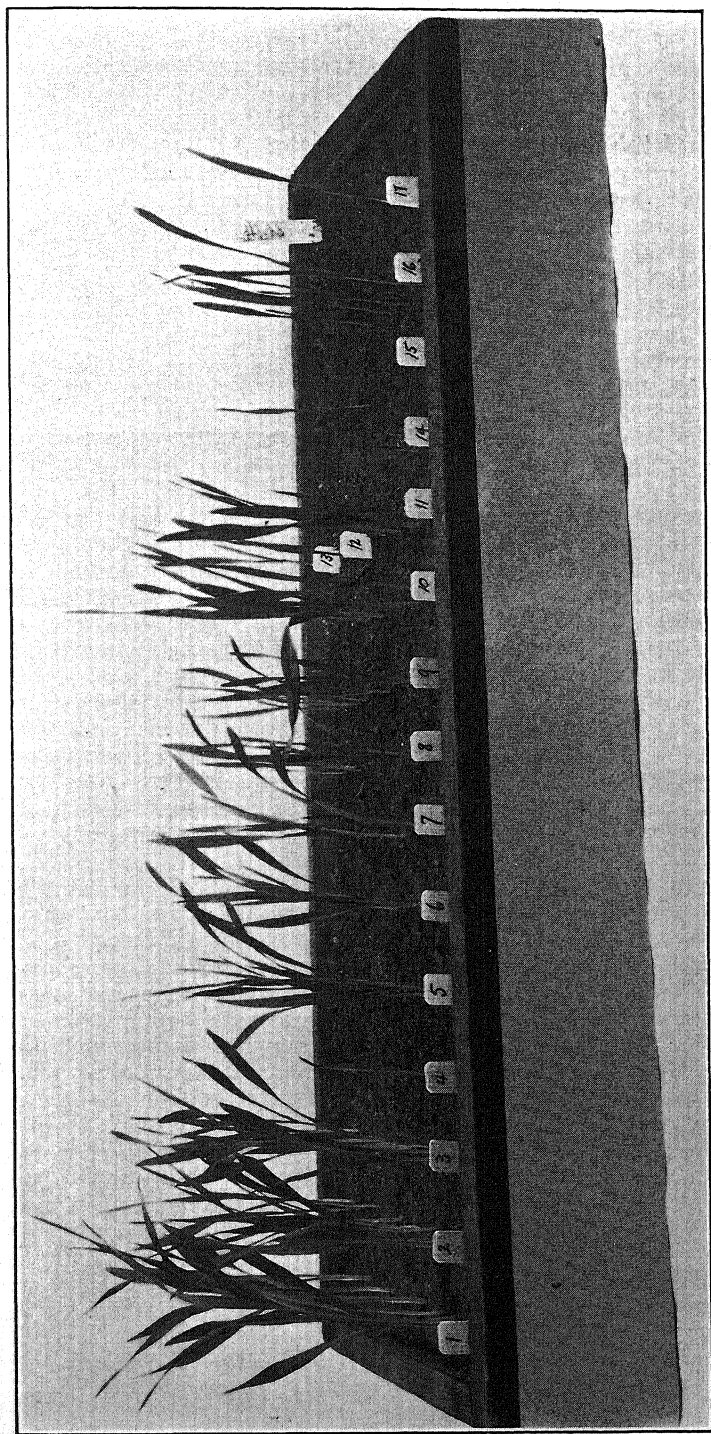


FIGURE 3.—Seedlings 12 days old from seeds produced by pollen and ovules that had undergone cold storage as described in table 8.

DISCUSSION

The spike of barley is treated quite severely in the emasculation process. The boot leaf is cut away just under the spike, the lateral kernels are pulled off, and the lemma and palet are cut off just above the tips of the unextruded anthers, which are removed with forceps. A glassine bag slipped over the spike helps to prevent excessive transpiration. Nevertheless, under good conditions, the seed set in carefully prepared cross-pollinated flowers is high. Thus, during the summer of 1938, at the Aberdeen substation of the Idaho Agricultural Experiment Station, H. V. Harlan and Mary L. Martini³ emasculated and pollinated a total of 8,085 barley florets, using over 100 varieties as male parents. These florets produced 6,154 seeds, or a set of 76.1 percent. Atlas, the best female parent, gave a 90.5 percent set on 1,402 flowers.

A good set of seed also is obtained when a culm is cut from the plant, the cut end is placed in distilled water, and the flowers are emasculated and pollinated. Four spikes treated in this way in 1934 yielded a set of 81.9 percent.⁴ In the present study, with 6 spikes a much lower set (52.7 percent) was obtained. The subjecting of such an excised, emasculated spike to cold storage doubtless increases the hazards of hybridization. Normal growth and development are interrupted, and life processes are greatly slowed down. The flowers of the emasculated spike in cold storage open slowly, remain open for several days, and then close with the stigma hairs beginning to wither at the tips. The anther filaments seldom elongate, and dehiscing anthers are relatively rare. Under such conditions there is much difficulty in getting viable pollen. Whole spikes may die without setting seed, and some flowers set seeds that develop a few days and then stop growing. Notwithstanding these difficulties, two spikes stored 19 days at 36° F. and dusted with pollen stored 8 days at the same temperature set 28 seeds in 36 florets, or 77.8 percent, and one of them set 16 seeds in 19 florets, or 84.2 percent.

The results herein recorded do not show a perfectly orderly decrease of viability in cold storage, as knowledge of the best stage for storage of such material was lacking and a rather wide range of stages was tried. It is possible that for best results emasculation should be done when the anthers are still green but after the spikes have attained considerable stiffness and the glumes have begun to green up. An untried suggestion is that spikes bearing their first-opened flowers be removed from storage at about the time the male parent is beginning to flower, and pollinated when a goodly number of the flowers have opened. Another suggestion is to emasculate all 6 rows of flowers, as a wider range of flower stages on the same spike would be obtained. As has been stated, the one spike to set seed after 42 days of storage was the only one of the 125 that had all 6 rows of flowers emasculated.

This technique makes it possible for plant breeders to obtain viable hybrid seed between early and late varieties of barley seeded at the same time or even between fall-sown and spring-sown sorts.

³ Oral communication.

⁴ POPE, MERRITT N. THE PRODUCTION OF BARLEY SEED BY POST-HARVEST POLLINATION. *Jour. Hered.* 28: 411-413, illus. 1935.

SUMMARY

Culms of barley with spikes, some emasculated and others unemasculated, were cut from the plant above the third node below the spike, and their cut ends were placed in vessels containing tap water. After cold storage at 36°, 40°, and 50° F. for various periods, samples were removed and their pollen and ovules tested as to their ability to produce seed.

Spikes excised and placed in cold storage within 3 days after emasculation had the highest set of seed after they were returned to the greenhouse.

Growth in length of culm practically ceased after 1 to 2 days in cold storage.

The elongation of the anther filament and the dehiscence of the anther were largely prevented in dark cold storage.

Flower opening and closing in emasculated spikes proceeded at a greatly reduced speed in cold storage, the rate being slowest at the lowest temperature.

Color in the culm was well preserved at 36° and less well preserved at 40° F. The culms stored at 50° were quite yellow on the nineteenth day and chlorotic on the twenty-sixth day.

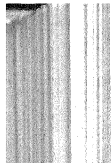
Unemasculated spikes set seed after 29 days of storage at 36° F., after 26 days at 40°, and after 14 days at 50°.

The extreme limit of storage for productive pollen was found to be 26 days at 36° F. and 19 days at 40° F. No pollen stored for 14 days or longer at 50° produced seed.

The extreme limit of storage found for production of seed from stored emasculated spikes was at least 42 days at 36° F., 29 days at 40°, and 14 days at 50°.

Seed from all the foregoing tests, except the single small seed produced on the emasculated spike stored for 29 days at 40° F., germinated and gave apparently normal plants after 8 weeks of growth.

Cold storage of undisturbed pollen or preferably of excised, emasculated spikes of barley makes it possible to obtain viable hybrid seed between varieties that normally flower on rather widely separated dates.



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EFFECTIVENESS OF HEAT PENETRATION IN THE CANNING OF MEAT IN THE HOME BY THE PRESSURE COOKER ¹

By CASPER I. NELSON, *bacteriologist and soil biologist*, and DOROTHY BERRIGAN, *formerly assistant in home economics research, North Dakota Agricultural Experiment Station*

INTRODUCTION

In a study of the effectiveness of heat penetration in meat canning sterility must be considered from two points of view: (1) Sterility in the strict laboratory sense, which implies absolute absence of all germ life. Numerous investigations show that there is a probability that this goal is seldom attained in canning meats. (2) Commercial sterility, which concerns those meats that keep and are safe for use over fairly long periods of storage. These products are termed sterile in a commercial sense.

It is conceivable that under the conditions of commercial sterility there are several possibilities: (1) Canned products which might not, be sterile in the strict laboratory sense but would keep in storage for a reasonable length of time; (2) conditions in which the organisms might still be present but only in a dormant stage; (3) conditions such that should the bacteria grow, they would be capable of causing spoilage only, and, not forming toxic substances, would offer no danger to the consumer.

When a whole carcass is to be canned and the many different parts are to be preserved, it is obviously necessary to alter the processing and packing to fit the cut and type of pack. Meat is a very poor conductor of heat, but convection currents in a fluid pack may permit a more rapid elevation of the temperature than in a solid type of pack. Clear juice or broth alone without the meat permits a more rapid circulation of heat in the container than when meat is present to interfere with the circulation. With these facts in mind and in order to make the results representative, the writers used various packs of meat such as are commonly employed in the home, and in exact duplicate.

The investigations of the factors controlling heat penetration were made for the purpose of determining their effect (1) in eliminating all dangerous organisms and (2) in controlling spoilage by the more persistent non-disease-producing organisms that are commonly found in canned meats.

EXPERIMENTAL PROCEDURE

In order to determine accurately, the internal temperature during the processing period, a 12-quart home type of pressure cooker similar to one described and illustrated by Magoon and Culpepper was used.² The equipment permits a long-stemmed thermometer to

¹ Received for publication February 24, 1939. This project was originally sponsored by the Federal Emergency Relief Administration following the drought years of 1934 and 1935 when stock breeders of the Dakotas and Montana had to dispose of their extensive herds because of the feed shortage. The writers herein acknowledge their gratitude and appreciation to the directors of the Federal Emergency Relief Administration for their valuable assistance in this work.

² MAGOON, C. A., and CULPEPPER, C. W. A STUDY OF THE FACTORS AFFECTING TEMPERATURE CHANGES IN THE CONTAINER DURING THE CANNING OF FRUITS AND VEGETABLES. U. S. Dept. Agr. Bul. 956, 55 pp., illus. 1931.

be inserted through the cover of the retort, directly through a brass plate soldered to the cover of the can, and into the center of the contents. The temperature can be read constantly and appropriate records made.

A group of organisms such as are most likely to occur in canned meats was chosen to test the effectiveness of the sterilizing process. This group consisted of *Clostridium botulinum*, *Escherichia coli*, a heat-resistant strain of *Streptococcus faecalis* isolated from a can of spoiled meat, and *Bacillus mesentericus*. *Cl. botulinum* (both strains A and B) was grown in a well-buffered proteose-peptone broth and aged 5 to 6 weeks, until the culture was almost entirely in the spore stage. It is well known that freshly-formed spores of *Cl. botulinum* possess little more resistance to heat than vegetative forms. Well-aged spore cultures were therefore required to represent the heat-resistant spore forms that are present in the soil and dust.

Escherichia coli is representative of the great group of non-spore-forming organisms found in the intestines of animals and is constantly associated with the organisms of infectious food poisoning. *E. coli*, being more generally resistant to conditions unfavorable to bacterial development, is a fair indicator for the survival of the pathogenic *Salmonella* varieties and other such food poisoners of animal intestinal origin.

From several cans of spoiled meat previously examined, there had been isolated a Gram-positive streptococcus which is quite heat resistant. Moist heat at 75° C. for 10 minutes is required to kill it. Cultural characteristics indicate its relationship to *Streptococcus faecalis*.³ All evidence indicated that this particular strain had appeared in the spoiled meat as a result of inadequate washing of carcasses at the time of slaughter and dressing. This streptococcus is frequently associated with *Escherichia coli* in meat spoilage, and, while not a gas producer, contributes to the fermentation of canned meat even under anaerobic conditions.

Bacillus mesentericus has been reported frequently in the spoilage of canned vegetables and meat where leaky cans are involved. The fact that it possesses a very heat-resistant spore suggested the use of this strict aerobe as an indicator of heat efficiency.

Various methods of introducing the organisms into the material to be canned were used. *Bacillus mesentericus* spores were dried on bits of sterile gauze which were placed in a pair of telescoping small test tubes. The disadvantage of this method is that the double walls of glass interfere with ready heat penetration and give high survival results. For this reason a suspension of spores in 1-percent saline in thin-walled, sealed glass ampoules was used. Such a device made it possible to place the organisms in a central position in the can where they could easily be found again. Suspensions of spores or vegetative cells in 0.5 cc. amounts were also injected directly into the body of the meat by hypodermic needle. Recovery of cultures was attempted from one of the duplicate cans immediately at the end of the process, and again from the contents of the second can at the end of the storage period. Meat, juice, and the contents of the ampoules were each tested separately with appropriate media under conditions of

³ This streptococcus grows well on ordinary media. It occurs in chains of three or four cocci—is Gram-positive, nonhemolytic, and produces a small amount of "greening" on fresh blood agar. It produces acid in lactose, levulose, mannite, salicin, saccharose, raffinose, and inulin. A facultative anaerobe and *Escherichia coli* were found associated with it in several cans of meat.

anaerobiosis, aerobiosis, or in carbohydrate media chosen to make quick identification possible.

Duplicate cans were stored for 2 to 5 months, which represents the average time of storage in the home. The temperature was maintained at about 35° C. This time and temperature represent conditions under which it is possible for spores of *Clostridium botulinum* to recover from the shock of heating and germinate. Delayed germination of spores is a possible danger. Dickson and his coworkers⁴ concluded that the spores of *Cl. botulinum* may remain dormant as long as 37 months after exposure to heat, and then germinate, multiply, and produce toxin. It has been shown recently⁵ that germination of spores of *Cl. botulinum* is inhibited at temperatures of 0° to 15° but is hastened at 30° to 35°. However, in no instance in the experiments reported here was growth of *Cl. botulinum* obtained from a stored can when it was not obtained from the first can also. In one case involving spores of *Bacillus mesentericus* a growth was obtained from a stored duplicate can when it was not obtained from the original one. In general, however, the duplicate cans checked perfectly.

The top cut of a round of beef was selected for use in the comparative canning tests because it consists of a tougher muscle which is usually preserved by canning methods, and also because it lends itself well to sectioning. The eye muscle of the round, when wrapped in a single layer of cheesecloth is easily located in the pack after processing and is large enough to allow one to place several prepared ampules in it as well as to inoculate it with a long hypodermic needle.

In packing the cans of meat, it was found that by having the top chuck cut into a slice three-fourths of an inch thick and again sectioned into pieces approximately 2½ inches square each pack was readily duplicated. This method also permitted the insertion of the thermometer into an identically placed piece of meat each time and obviated the possibility of the thermometer bulb recording liquid or space temperature rather than the temperature of the meat.

A number of packs varying in weight and size were processed. It was estimated that 495 gm. for No. 2, 725 gm. for No. 2½, and 885 gm. for No. 3 cans represented packs that allowed for sufficient head space in the can and gave the most desirable results. Three or more cans were used for each test of variation.

In order to allow for variation in initial temperatures and have all packs processed under as nearly identical conditions as possible, duplicate cans filled with the same weight of meat were preheated in a bath of boiling water which came to within 1 inch of the top of the cans. All cans were preheated until their contents registered 31° C. This rise in temperature usually took 1 to 40 minutes, depending on the size, weight, and nature of the contents as well as the temperature at which the meat was packed.

Duplicate cans were then sealed and the thermometer was inserted in the center of the can attached to the cover of the retort. One inch of boiling water was placed in the retort, the duplicate can was placed on the bottom of the cooker, the cover adjusted, and the apparatus sealed. After the retort was sealed the pet cock was left open for

⁴ DICKSON, ERNEST C., BURKE, GEORGINA S., BECK, DOROTHY, and JOHNSTON, JEAN. STUDIES ON THE THERMAL DEATH TIME OF SPORES OF *CLOSTRIDIUM BOTULINUM*. IV. THE RESISTANCE OF SPORES TO HEAT AND THE DORMANCY OR DELAYED GERMINATION OF SPORES WHICH HAVE BEEN SUBJECTED TO HEAT. *Jour. Infec. Diseases* 36: [472]-483. 1925.

⁵ TANNER, FRED W., and OGLESBY, ELAINE E. INFLUENCE OF TEMPERATURE ON GROWTH AND TOXIN PRODUCTION BY *CLOSTRIDIUM BOTULINUM*. *Food Res.* 1: 481-494. 1936.

7 minutes to allow the enclosed air to escape. The pet cock was then closed and the temperature of the meat was recorded at 1-minute intervals. The processing time was calculated from the time the retort attained the desired pressure. The retort was then removed from the heating element, the pet cock opened, and the steam fully released. The bulged cans were then cooled by one of two methods: (1) By being allowed to remain at room temperature until quite cool, or (2) by being plunged into cold running tap water at approximately 10° C.

Examination of the contents of the can in which the thermometer had been placed was made on the day of canning, the duplicate can being stored. At the end of the storage period, the contents were again checked for keeping quality and for evidence of bacterial growth or survival.

EXPERIMENTAL DATA

Graphs were prepared from the temperature readings made as described. So far as possible only one variable at a time was permitted in these experiments. The chief variables were: (1) Type of pack; (2) steam pressure (i. e., 10 pounds and 15 pounds); (3) can size; (4) duration of process; and (5) methods of cooling (i. e., cold dipping and room-temperature cooling). While the size of the apparatus permitted only two cans at a time to be processed each variation in the experiments was repeated three or more times to make sure that results were capable of duplication. The data closest to the average for the series of trials were used in the following graphs.

For comparison, the maximum temperature attainable under 10 pounds and 15 pounds of steam pressure is indicated by heavy horizontal lines. This is the ceiling or upper limit of temperature attainable in the can at the pressure used. The crest of the curve entering the zone between 100° C. and the ceiling defines the dome of actual sterilization, a product of temperature and time. The area of the dome within this zone marks the actual temperature-time-sterilization in the particular experimental case. The efficiency (i. e., effective heat penetration) of any arrangement of the equipment is expressed as a percentage upon each curve. This percentage is the ratio of the area within the dome to that of the theoretical sterilizing zone within the time limits.

Bacteria survival tests at different temperatures are also imposed upon the curves to indicate the probable effectiveness of the process. These results may or may not be consistent with the percentage results.

In figure 1 it may be seen that heat penetration is readiest where the least interference with convection currents is met, that is, in the order: (1) Liquid; (2) ground-meat patties; (3) raw cubed beef; and (4) raw ground meat, solid pack. Though the nutrient broth was processed at only 10 pounds pressure, it heated so effectively that sterilization was accomplished, which was not true of all the other packs at a higher steam pressure.

Under 15 pounds pressure absolute sterility was not assured, but the ever dangerous *Clostridium botulinum* and the intestinal organism *Escherichia coli* were destroyed even when the efficiency ratios were low. The surviving *Bacillus mesentericus* is a strict aerobic organism,

and cannot grow in a perfectly sealed can. In "leakers" it can grow and cause very apparent spoilage. It is exceedingly difficult to eliminate the spores of this soil organism.

Two curves representing identical cans, but with one at 15 pounds pressure and the other at 10 pounds pressure, show a wide difference in heat penetration (fig. 2). The difference is sufficient to account for differences in bacterial results.

Heat penetration as influenced by variation in can size is shown in figure 3; no effort was made to represent any variation in can area by the use of cans of different shapes. A close correlation between heat penetration and can size is indicated. As in figure 1, *Bacillus mesentericus* spores survived but other organisms were killed.

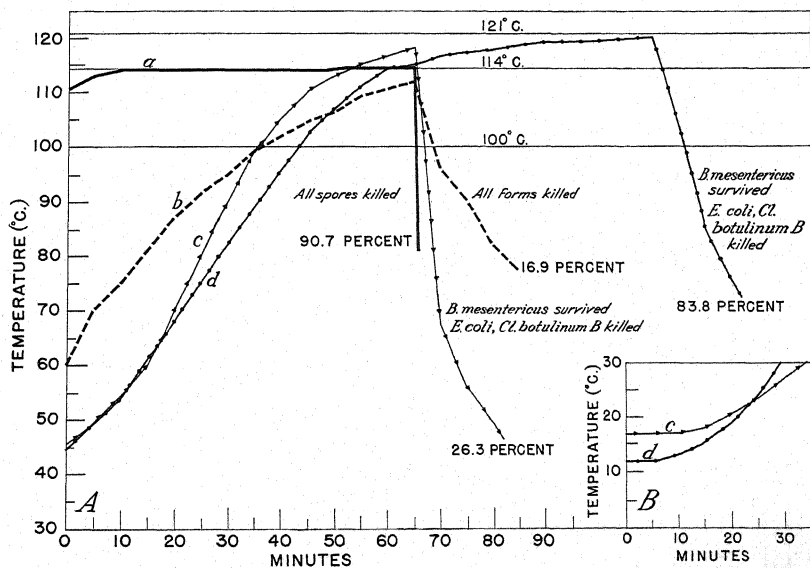


FIGURE 1.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), caused by type of pack: A, Heating period; B, pre-heating period: a, 725 cc. of nutrient broth processed at 10 pounds pressure in No. 3 can; b, 725 gm. of pan-fried beef patties processed at 15 pounds pressure in No. 3 can; c, 885 gm. of raw cubed beef processed at 15 pounds pressure in No. 3 can; d, 885 gm. of raw ground beef processed at 15 pounds pressure in No. 3 can.

In figure 4, which shows the time variable, maximum heat penetration is seen to have been reached at 90 minutes. This series of trials was run to determine the feasibility of continuing the process until actual sterility was attained. Actual sterility was attained in 90 minutes. As shown in the other figures, *Escherichia coli* and *Clostridium botulinum* spores do not survive the 60-minute period in No. 2½ cans at 15 pounds pressure. This, then, indicates a threshold of safety at 65 minutes under these conditions of can size and pressure. It indicates also the possibility of actual sterility at 90 minutes, but the necessity is not shown.

The fact is self-evident that cooling at room temperature prolongs the sterilization process within the can. During the cooling process

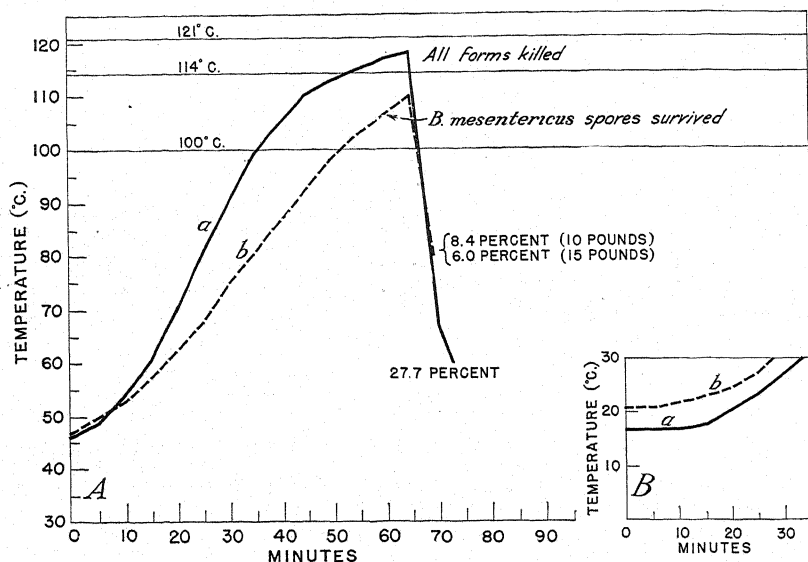


FIGURE 2.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), caused by differences in steam pressure while processing: A, Heating period; B, preheating period; 725 gm. of raw cubed beef processed at 15 (a) and at 10 (b) pounds pressure for 65 minutes in No. 2½ cans.

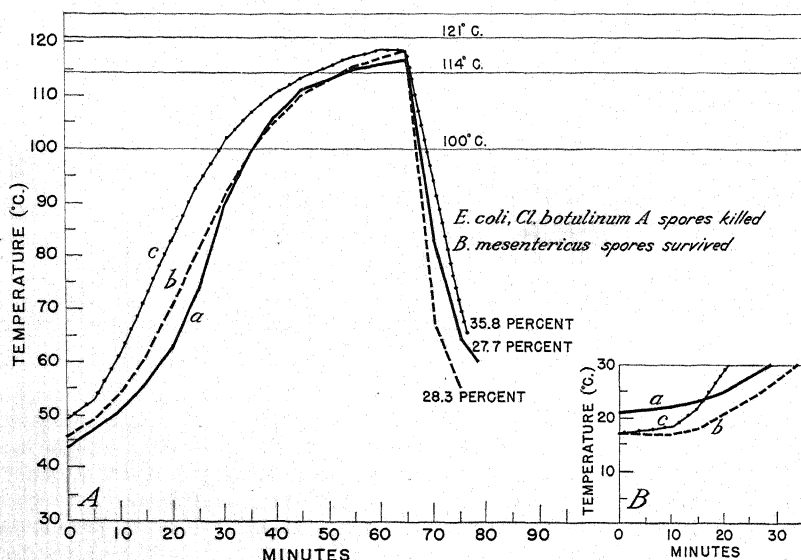


FIGURE 3.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), caused by differences in can size in processing raw cubed beef at 15 pounds pressure for 65 minutes: A, Heating period; B, preheating period: a, 885 gm. in a No. 3 can; b, 725 gm. in a No. 2½ can; c, 495 gm. in a No. 2 can.

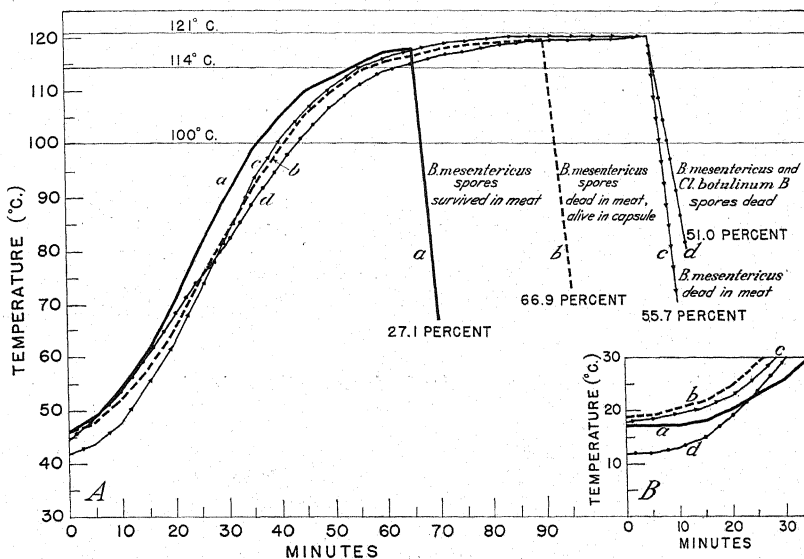


FIGURE 4.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), caused by differences in time of heating at 15 pounds pressure in No. 2½ cans: A, Heating period; B, preheating period: a, 725 gm. of raw cubed beef containing *Bacillus mesentericus* heated for 65 minutes; b, 725 gm. of raw cubed beef containing *B. mesentericus* heated for 90 minutes; c, 725 gm. of raw cubed beef containing *B. mesentericus* heated for 110 minutes; d, 725 gm. of raw cubed beef containing *B. mesentericus* and *Clostridium botulinum*, B strain, heated for 110 minutes.

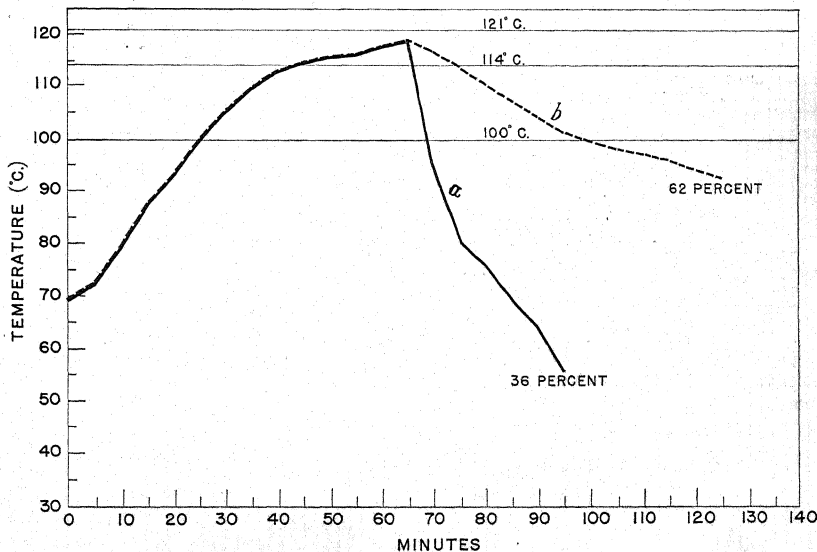


FIGURE 5.—Variations in effective heat penetration, shown as percentage (see text for fuller explanation), as related to method of cooling: 725 gm. of raw cubed beef processed for 65 minutes at 15 pounds pressure in a No. 2½ can (a) cold-dipped and (b) left at room temperature.

cooking also progresses (fig. 5). The use of room-temperature cooling may well make it feasible to decrease the period of processing or the pressure needed to pass the threshold of safety. Bacterial test results were consistent with the percentage efficiency.

In addition to the five variables represented in figures 1 to 5 there is another factor which has proved important in the sterilization of meat in the can. This is the temperature of the meat upon its receipt as modified by the preheating period. As shown in table 1, the temperature increase during the preheating period is very slight during the first 5 minutes but accelerates constantly in the following 5-minute periods. If the preheating period is extended to 20 minutes or longer, the temperature difference between meats of different temperatures is eliminated.

TABLE 1.—*Temperature rise in successive 5 minutes of the preheating period of canned round of beef, later processed for 65 minutes at 15 pounds pressure, as related to initial temperature of the meat*

Process ¹	Initial temperature	Temperature at the end of—				Length of processing	Internal temperature on removal from can	Comments
		5 minutes	10 minutes	15 minutes	20 minutes			
Steamer-exhausted.....	°C. 8	°C. 9	°C. 12	°C. 20	°C. 29¾	Minutes 65	°C. 95	1 inch of water in steamer for exhausting.
Pan-seared.....	8	-----	-----	-----	-----	65	99	Internal temperature 57°-65° C. when packed into cans; 94° C. when cover was removed.
Seared in oven; preheated to 500° F.	10	14	16	22	{ 23 24 30 }	65	98	93° C. when cover was removed (salt omitted).
No exhausting; packed cold.	10	-----	-----	-----	-----	65	98¾	Expansion sounds at 15 minutes; 91° C. on opening.
Surplus meat exhausted 20 minutes; water 1 inch from top of cans.	10	16	19	25	{ 30 33 }	55	97	Length of time cut 10 minutes; more water in steamer for exhausting.

¹ 24 pounds of beef round as received. Meat wiped with damp cloth, sliced approximately ¾ inch thick and into pieces 2½ inches square; ¼ teaspoon of salt added to each can. No. 2 cans used. Average weight when packed 1¼ pounds. 3 cans used in each test: 1 can plunged into cold water immediately upon removal from pressure cooker; 1 can opened immediately and internal temperature taken; 1 can left at room temperature without cooling. On opening, all meat seemed stringy and appeared overcooked.

Table 2 reports the same type of experiments with beef received frozen (average temperature -2° C.) and placed in cans only after it had thawed out. During the fourth 5-minute interval of preheating the temperatures reached were not so uniform as those in table 1. This would seem to imply that the differences would be eliminated in still later 5-minute periods.

TABLE 2.—*Temperature rise in successive 5 minutes of the preheating period of canned sirloin of beef, received frozen, and steamer-processed¹ at 15 pounds pressure for different periods of time*

Initial temperature ° C.	Temperature at the end of—				Length of processing	Time for gage to reach 15 pounds	Internal temperature on removal	Comments
	5 minutes	10 minutes	15 minutes	20 minutes				
6.....	6	12	23	31	45	3	97	Expansion sounds at 10, 16, 21 minutes; 2 at 26 minutes; 95° C. on opening.
7.....	7	9	18	23				
7.....	7	7	12	17				
1.....	1	1	1	7	40	25½	98	Expansion sounds at 6, 11, 14, 21, 33 minutes; 90° C. on opening.
0.....	0	1	3	11				
0.....	0	3	7	14				
8.....	8	12	20	29	30	3	96	Expansion sounds at 10, 11 minutes; 2 at 13, 14, and 14½ minutes; 91° C. on opening.
4.....	4	13	14	17				
9.....	11	18	25	32				
11.....	15	17	20	25	20	3	84	Expansion sounds at 2, 3, 14 minutes; 4 minutes, and 20 minutes, as pressure was being released.
13.....	14	20	27	34				
10.....	10	12	17	23				

¹ 17½ pounds sirloin of beef received frozen, average temperature -2° C. Left at room temperature until approximately 0° C. Meat wiped with damp cloth and sliced ¾ by 2¼ by 2¼ inches. 1¼ pounds total weight of packed No. 2 cans. All cans exhausted 20 minutes in 1 inch of boiling water. Thermometers inserted into center of meat and temperatures taken each 5-minute period of exhausting. 3 cans used in each test: 1 can opened immediately on removal from pressure cooker; 1 can dipped into cold water until ends contracted; 1 can left at room temperature. All cans labeled and stored at room temperature.

From these observations, it appeared that the setting of an arbitrary period for preheating was not sufficiently accurate for this type of work. Therefore, in all the experiments reported the steam processing began only when the preheating had produced a temperature of 31° C.

SUMMARY AND CONCLUSIONS

In solid meat packs where convection currents are not possible, temperatures reached under 10 pounds of steam are insufficient to destroy such bacteria as were tested in this experiment. Sixty-five minutes at 15 pounds pressure (121° C.) appeared to be the minimum time and temperature that could safely be allowed for sterilization of No. 2 cans, 90 minutes for No. 2½ cans, and 110 minutes for No. 3 cans.

Cold dipping proved to be the most desirable means of treating the cans after they had been removed from the retort. This process reduces the cooking temperature at once and so eliminates the danger of overcooking and at the same time reduces the pressure exerted on the seal. Leaving the processed cans at room temperature prolongs the already long cooking period, gives an overcooked product, and because of the unrelieved internal steam pressure, strains the seal.

Clostridium botulinum spores of both strains A and B were destroyed even when the heat efficiency was low. When the processing time was extended over a period of at least 65 minutes at 15 pounds pressure the elimination of food poisoning organisms was certain. It was found, however, that the temperatures attained at even the higher pressure level were not uniformly effective in destroying thermophilic organisms such as *Bacillus mesentericus* and *Streptococcus faecalis*.

Heat penetration is readiest where the least interference with convection currents is met, in the order: (1) Liquid; (2) chunk meat; (3) ground-meat patties; (4) ground meat, solid pack; and (5) solid chunk. The probability exists that certain heat-resistant, putrefactive aerobes will survive the practical limit of prolonged processing beyond which the product deteriorates. This may also be true of putrefactive spore-bearing anaerobes.

Because of the lag in the heat curves, the ratio of efficiency is surprisingly low. The gradient of the noneffective part of the curve varies in the order of the packs just given.

So far as the experiments have been carried, 15 pounds pressure is indicated as necessary for sterility in the laboratory sense. Within that pressure limit the time variable should be made to suit the type of pack and size of container. In the canning of beef in tins where 15 pounds steam pressure is used over periods of time dependent upon the variation in procedure, the threshold of safety has been reached and probably passed. The term "safety" as here used refers to freedom from food poisoning by *Clostridium botulinum* and to infectious food poisoning by the *Salmonella* group and allied intestinal organisms. By careful control of still other factors which influence rapidity of heat distribution in the can, such as fluid and fat, it would undoubtedly be possible to process meat just under the threshold of safety and still have a product that would keep for a reasonable length of time.

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No. 7

KNOB POSITIONS ON CORN CHROMOSOMES¹

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United States Department of Agriculture*

INTRODUCTION

Chromosome knobs are enlargements on the chromosomes of corn (*Zea mays* L.) and its near relatives, visible on the threads at prophase of the first meiotic division. These enlargements stain deeply with carmine and vary in size from swellings but slightly more prominent than the adjacent chromomeres to those that equal the volume of a whole chromosome at anaphase.

McClintock² was the first to report knobs on the midprophase chromosomes of corn. Since this first report, several investigators have found that knobs on the chromosomes of corn are seemingly a constant feature and aid materially in the identification of many of the 10 chromosomes.

The writer was impressed by the fact that the location of the various knobs was not at random and that certain knobs are present in nearly all plants examined, whereas others are present in a very few plants. The location of the knobs and the unequal frequency with which they occur on the 20 arms of the chromosomes suggested the possibility that their frequency might be related to their position.

MATERIAL AND METHODS

The data for both knob frequency and knob position were obtained from camera lucida drawings, made at a magnification of $\times 3,000$, of chromosomes in pollen mother cells of the 33 Indian varieties of corn from the United States that were described in a previous publication,³ and a similar collection of 41 varieties from isolated regions of Mexico.

The usual carmine-smear technique for preparing pollen mother cells was used. The cells were pressed to flatten the chromosome threads as much as possible without rupturing the cells. All measurements are given in microns, and the slight error, due to the fact that chromosomes were not always brought into one plane throughout their whole length, has been neglected because corrections are tedious and would seem to affect the results very little.

LENGTH OF THE CHROMOSOMES

The length of corn chromosomes is found to vary in pollen mother cells, depending on the stage of development. Measurements in the

¹ Received for publication July 24, 1939.

² MCCLINTOCK, BARBARA. A CYTOLOGICAL DEMONSTRATION OF THE LOCATION OF AN INTERCHANGE BETWEEN TWO NONHOMOLOGOUS CHROMOSOMES OF *ZEA MAYS*. Natl. Acad. Sci. Proc. 16: 791-796, illus. 1930.

³ LONGLEY, A. E. CHROMOSOMES OF MAIZE FROM NORTH AMERICAN INDIANS. Jour. Agr. Res. 56: 177-195, illus. 1938.

present study have been restricted to those figures in which the chromosome threads are much extended. It has been found that chromosomes contracted to less than one-half their length at the most extended stage are not satisfactory for observing the various characteristic markings on the threads. The smaller knobs, for example, in much-contracted threads are not sharply differentiated from the normal thickening of the thread which results from the contraction.

The measurements of all chromosomes in 28 different cells from 14 Indian varieties are given in table 1; only two of these measurements are from the same plant. This small group of measurements represents the general results of measuring the length of corn chromosomes and clearly demonstrates that chromosome identification must rely on features other than relative length, such as the fiber-attachment position, the presence of knobs, and other less prominent characteristics.

TABLE 1.—Length of midprophase chromosomes of corn from 28 pollen mother cells

Cell No.	Length of chromosome No. —									
	I	II	III	IV	V	VI	VII	VIII	IX	X
1.....	80.00	80.83	79.17	73.33	72.50	65.00	54.17	52.50	50.00	42.07
2.....	93.33	68.33	75.83	67.50	56.67	50.00	45.83	52.50	50.00	44.10
3.....	98.33	76.67	77.50	60.83	75.00	53.33	60.83	59.17	62.50	46.62
4.....	76.67	62.50	57.50	60.00	61.67	49.17	54.17	36.67	46.67	41.18
5.....	80.83	78.33	73.33	73.33	56.67	47.50	49.17	59.17	50.83	43.33
6.....	100.67	77.50	95.83	63.33	94.17	55.83	64.17	45.00	72.50	52.75
7.....	85.83	70.00	69.17	54.17	58.33	47.50	49.17	43.33	42.50	35.16
8.....	84.17	64.17	70.83	66.67	70.83	48.33	47.50	50.83	52.50	46.29
9.....	83.33	79.17	65.83	66.67	59.17	39.17	39.17	49.17	38.33	31.77
10.....	51.67	35.00	34.17	31.67	33.33	30.00	25.00	28.33	39.17	32.02
11.....	50.83	45.83	44.17	44.17	38.33	36.67	33.33	36.67	30.83	27.98
12.....	87.50	82.50	60.00	67.50	56.67	47.50	42.50	38.33	51.67	40.41
13.....	83.33	63.33	63.33	60.00	65.83	38.33	37.50	42.50	42.50	36.85
14.....	72.50	61.67	62.50	55.00	61.67	37.50	45.83	44.17	40.83	33.97
15.....	54.17	41.67	40.00	38.33	40.83	34.17	30.00	30.00	29.17	27.73
16.....	84.17	64.17	52.50	66.67	53.33	35.00	50.00	45.83	36.67	35.13
17.....	72.50	61.67	53.33	49.17	52.50	38.33	42.50	40.83	40.00	36.20
18.....	140.00	93.33	95.83	87.50	89.17	82.50	81.67	77.50	56.67	46.16
19.....	87.50	66.67	67.50	64.17	66.67	54.17	45.00	49.17	39.17	32.79
20.....	55.00	42.50	40.83	40.83	37.50	31.67	32.50	33.33	30.83	30.01
21.....	54.17	37.50	33.33	32.50	34.17	29.17	29.17	30.00	30.83	26.45
22.....	57.50	35.83	42.50	35.83	35.83	39.17	26.67	26.67	24.17	25.00
23 ¹	67.50	57.50	52.50	50.00	52.50	38.33	36.67	39.17	39.17	33.23
24 ¹	70.00	60.83	53.33	41.67	44.17	34.17	31.67	35.83	31.67	27.53
25.....	92.50	76.67	71.67	58.33	57.50	49.17	44.17	46.67	52.50	44.62
26.....	69.17	52.50	62.50	52.50	45.00	36.67	35.83	40.00	28.33	25.74
27.....	63.33	44.17	43.33	38.33	40.00	30.00	32.50	39.17	31.67	28.96
28.....	90.00	110.00	99.17	73.33	84.17	70.83	60.83	65.83	63.33	51.54
Mean.....	78.09	63.96	62.05	56.19	56.93	44.61	43.84	44.23	43.04	36.63

¹ Cells 23 and 24 were from the same plant.

Table 2 presents separately the mean lengths of the 10 chromosomes of two corn groups and then combines these groups—the United States Indian varieties and the Mexican varieties—to give a general mean for each chromosome length. It is clear from the means of tables 1 and 2 that there is a general reduction in the length of the chromosomes from I to X but that chromosomes V and VIII are on an average slightly longer than chromosomes IV and VII, respectively.

The length of a chromosome seems never to be fixed. In the first meiotic prophase there is a gradual shortening in length until the metaphase is reached. Consequently, the measurements reported in tables

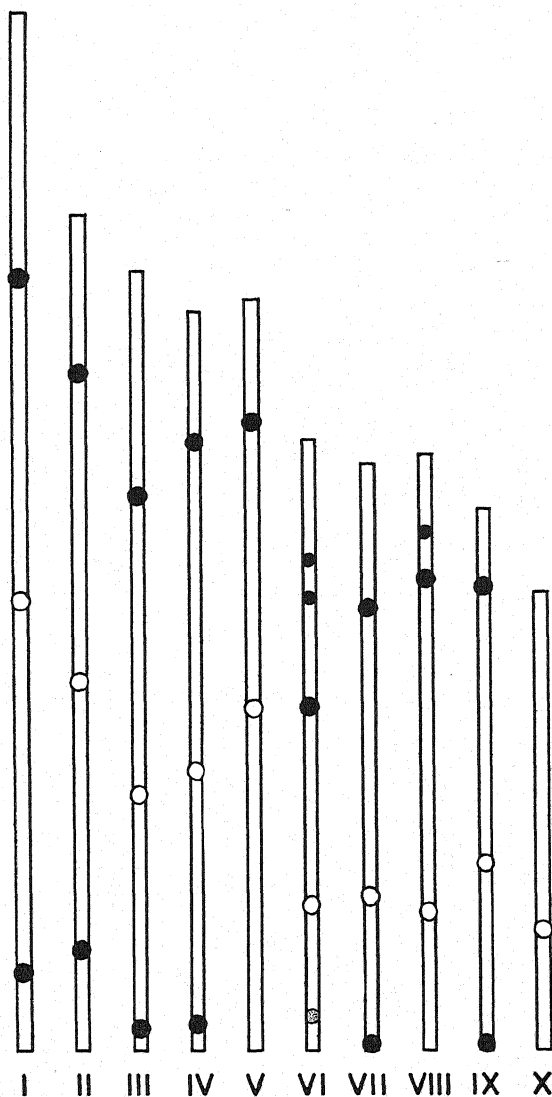


FIGURE 1.—Diagram of the 10 chromosomes of corn, showing knobs (black) and fiber attachments (circles). $\times 1,500$.

1 and 2 are given to show relative lengths only at that phase when knobs are most prominent and their positions most readily fixed. Figure 1 shows diagrammatically the relative lengths, as given in the last column of table 2, of all 10 chromosomes.

TABLE 2.—Mean length of midprophase corn chromosomes

Chromosome No.	United States Indian varieties		Mexican varieties		Combined United States Indian and Mexican varieties	
	Measurements	Mean length	Measurements	Mean length	Measurements	Mean length
	Number	μ	Number	μ	Number	μ
I.....	250	82.83	93	81.22	343	82.40
II.....	218	66.32	88	66.95	306	66.50
III.....	262	61.62	92	63.09	354	62.00
IV.....	240	58.72	102	58.91	342	58.78
V.....	240	60.08	80	59.05	320	59.82
VI.....	270	49.33	107	47.23	377	48.73
VII.....	261	46.71	106	46.96	367	46.78
VIII.....	258	47.21	103	48.16	361	47.45
IX.....	313	42.79	103	44.59	416	43.24
X.....	279	37.07	112	36.59	391	36.93

LENGTH OF ARMS

The fiber attachment divides each chromosome into two arms. In this study of knob position, each arm is considered independently of the length of the whole chromosome and in consequence the mean lengths of the 20 arms of the 10 chromosomes, given in table 3, are more relevant to the problem under discussion than are the chromosome lengths given in tables 1 and 2. In table 3 the chromosome arms are arranged in order of length, beginning with the shortest and ending with the longest. The number of measurements is the same as in table 2 in each case. Figure 2 shows diagrammatically the different lengths of the 20 arms, as given in the last column of table 3.

TABLE 3.—Mean length of arms of midprophase chromosomes of corn

Chromosome		Length of arm of—			Chromosome		Length of arm of—		
No.	Arm	United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	No.	Arm	United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
X.....	Short	μ 9.76	μ 9.93	μ 9.81	II.....	Short	μ 29.31	μ 30.01	μ 29.51
VIII.....	do	11.09	11.69	11.26	V.....	Long	32.78	31.44	32.45
VI.....	do	11.68	12.50	11.91	VII.....	do	34.18	34.72	34.34
VII.....	do	12.53	12.23	12.44	I.....	Short	36.15	35.14	35.87
IX.....	do	15.01	15.81	15.21	VIII.....	Long	36.12	36.47	36.22
III.....	do	20.46	20.67	20.51	IV.....	do	36.03	36.96	36.31
IV.....	do	22.69	21.95	22.47	VI.....	do	37.65	34.73	36.82
X.....	Long	27.31	26.66	27.12	II.....	do	37.01	36.88	36.97
V.....	Short	27.29	27.61	27.37	III.....	do	41.16	42.42	41.49
IX.....	Long	27.78	28.77	28.03	I.....	do	46.69	46.09	46.52

KNOB POSITIONS

Knobs have been found on all but 5 of the 20 arms of the 10 chromosomes, disregarding the knoblike enlargement comprising the nucleolus "organizer" in the short arm of chromosome VI. Thirteen arms apparently have only 1 knob each, while 1 of the 2 remaining arms may have 2 knobs and the other may have 3.

Two convenient ways of expressing the position of a knob are as its distance from the end of the arm and as its distance from the fiber attachment. Table 4 shows the mean position of all knobs expressed in these two ways, and figure 2 shows diagrammatically the mean positions of all 18 knobs.

CHROMOSOME

NO. ARM

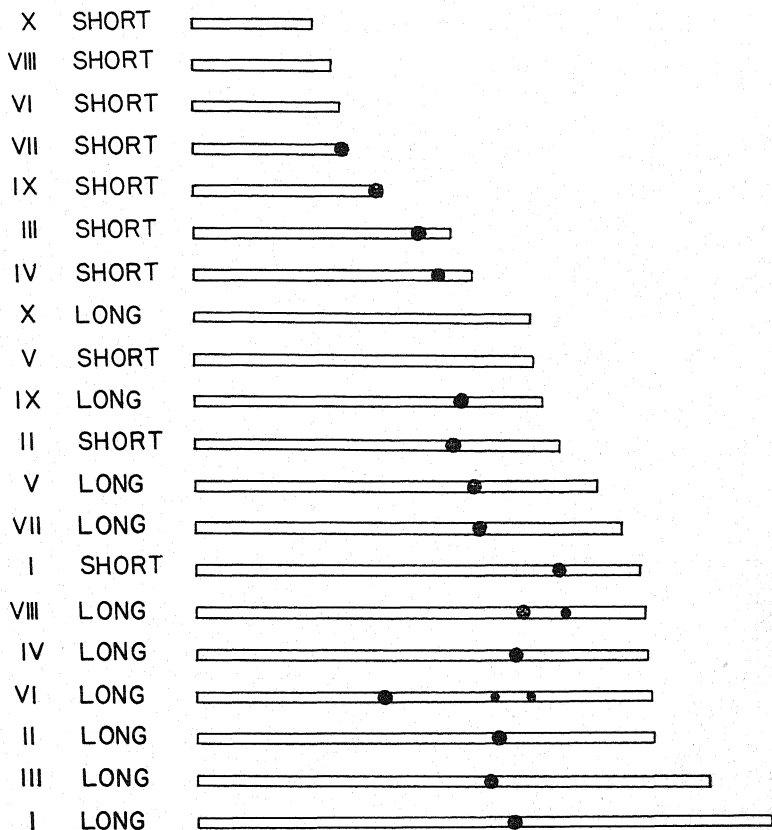


FIGURE 2.—Diagram of the 20 chromosome arms of corn, arranged in order of their length and showing knobs (black), the left end of each arm being the fiber-attachment point. $\times 1,500$.

Although these 18 knobs differ widely in the frequency of their occurrence, each deviates very little from its mean position; therefore, all students of corn chromosomes think of each knob as being constant in position. The fact that a knob is in the same position on the chromosome, even in plants coming from widely separated localities, suggested that there are 18 points on the 20 chromosome arms having the ability to form a knob under certain conditions. These points doubtless exist on the chromosome threads of all corn plants but are visible only when knobs are formed. Consequently, the data have

been examined to determine whether the frequency with which a knob is found depends on its position on the chromosome thread.

TABLE 4.—Mean distance of knobs from fiber attachment and from end of chromosome arms

Knob location on chromosome—		United States Indian varieties		Mexican varieties		Combined United States Indian and Mexican varieties	
		Distance of knob from—		Distance of knob from—		Distance of knob from—	
No.	Arm	End	Fiber attachment	End	Fiber attachment	End	Fiber attachment
VII	Short	0.00	12.52	0.00	12.23	0.00	12.44
IX	do	0.00	15.01	0.00	15.81	0.00	15.21
III	do	2.25	18.21	1.50	19.17	2.00	18.51
IV	do	3.00	19.69	2.17	19.78	2.45	20.01
IX	Long	5.64	22.15	7.63	21.11	6.25	21.78
II	Short	8.75	20.56	8.16	21.86	8.53	20.99
V	Long	9.53	22.95	9.56	21.88	9.75	22.70
VII	do	11.35	22.83	11.39	23.33	11.37	22.97
I	Short	6.24	29.91	6.71	28.43	6.46	29.41
VIIIa ¹	Long	5.36	30.76	6.67	29.81	6.24	29.98
VIIIb ²	do	9.46	26.66	10.50	25.98	9.79	26.43
IV	do	10.33	25.70	10.36	26.60	10.35	25.96
VIa ¹	do	9.89	27.77	9.08	25.65	9.64	27.18
VIb ¹	do	12.77	24.89	12.38	22.85	12.65	24.17
VIc ³	do	21.84	16.31	20.86	13.87	21.29	13.53
II	do	13.03	23.97	11.87	25.01	12.63	24.35
III	do	17.28	23.89	18.65	23.77	17.84	23.65
I	do	19.88	26.81	23.33	22.76	20.91	25.61

¹ Knob nearest the end.

² Knob in second position from the end.

³ Knob in third position from the end.

KNOB FREQUENCY

The number of knobs on the chromosomes may be the same in sister plants of a variety, but usually in varieties that have not been inbred the number varies somewhat from plant to plant, and this variation is frequently very pronounced when plants of varieties from different geographical regions are compared.

Not all knobs occur with the same frequency. Some knobs are usually present, whereas others are infrequently found.

The differences in frequencies led to an attempt to measure the frequency of occurrence of each knob in all the corn material studied. Since the data included a greater number of plants from varieties with few knobs on their chromosomes than from varieties with many knobs, it seemed best to group all plants according to the number of knobs present. This arrangement gave a grouping with classes ranging from 1 to 14. From this grouping the frequency of occurrence of each knob in each group was determined. The 14 determinations for each knob were then averaged to find the frequency of each knob in the whole population. Table 5 shows the frequencies determined by this method. This population does not include plants without knobs, but there were less than 1 percent of such plants.

TABLE 5.—Percentage frequency of the 18 different chromosome knobs

Chromosome		Percentage frequency in indicated number of plants having—														
No.	Arm	14 knobs (4) ¹	13 knobs (16)	12 knobs (23)	11 knobs (25)	10 knobs (23)	9 knobs (17)	8 knobs (11)	7 knobs (23)	6 knobs (25)	5 knobs (16)	4 knobs (24)	3 knobs (37)	2 knobs (75)	1 knob (26)	Mean
		<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
I	Short	100.0	100.0	100.0	92.0	95.7	100.0	100.0	87.0	72.0	75.0	83.4	87.7	96.0	69.2	89.3
V	Long	100.0	100.0	100.0	96.0	100.0	82.4	81.8	87.0	68.0	55.3	29.2	40.2	12.0	11.5	68.8
VII	do	100.0	100.0	100.0	92.0	91.3	76.5	90.9	69.6	76.0	43.7	16.7	14.5	9.3	3.8	63.2
IV	do	100.0	100.0	100.0	100.0	91.3	100.0	90.9	66.5	56.0	50.0	20.8	7.0	5.3	0	62.7
VIIa ²	do	100.0	81.2	79.1	80.0	95.7	52.9	27.3	43.5	40.0	50.0	75.0	59.7	48.0	15.3	60.5
II	do	100.0	100.0	83.3	100.0	78.3	82.4	72.7	47.8	56.0	37.5	37.5	22.8	5.3	0	58.8
VIIIb ³	do	100.0	100.0	100.0	100.0	69.6	70.5	63.6	65.2	60.0	50.0	16.7	12.3	2.7	0	57.9
III	do	100.0	100.0	100.0	92.0	91.3	76.5	90.9	47.8	60.0	6.3	8.3	1.7	1.3	0	54.0
VIIb ³	do	75.0	81.2	83.3	60.0	73.9	47.0	36.4	39.1	24.0	25.0	58.3	21.0	10.7	0	45.3
II	Short	100.0	68.7	79.1	72.0	60.8	35.3	55.5	43.5	48.0	12.5	16.7	26.3	10.7	0	44.9
I	do	75.0	93.8	65.2	72.0	78.3	58.8	18.2	39.1	20.0	18.7	12.5	0	0	0	39.4
I	Long	50.0	68.7	69.6	48.0	13.0	41.2	18.2	21.7	12.0	6.3	0	0	0	0	24.9
VIIIa ²	do	75.0	43.7	45.8	48.0	30.4	47.0	18.2	13.0	8.0	0	0	0	0	0	23.5
IX	do	100.0	100.0	50.0	4.0	8.7	11.8	9.1	4.3	0	12.5	8.3	1.7	0	0	22.2
III	Short	75.0	81.3	16.7	12.0	8.7	17.6	9.1	13.0	16.0	6.3	0	0	0	0	14.7
VIe ⁴	Long	50.0	56.3	20.9	16.0	13.0	0	0	4.3	16.0	6.3	12.5	0	0	0	13.9
IV	Short	0	12.5	0	12.0	4.3	0	27.3	17.4	12.0	6.3	8.3	1.7	1.3	0	7.4
VII	do	0	0	4.2	4.0	0	0	0	0	0	0	0	0	0	0	.6

¹ Numbers in parentheses indicate number of plants.² Knob nearest to the end.³ Knob in second position from the end.⁴ Knob in third position from the end.

KNOB-BEARING REGIONS

A few minutes' study of the 18 knobs shown on the chromosomes of figure 2 will be sufficient to convince the reader that they are not distributed uniformly over the whole length of the threads. There is an appreciable piece of each chromosome adjacent to the fiber attachment that is knobless. The fact that all knobs are found at some distance from the fiber attachment suggested that an attempt be made to determine the length of that portion on each chromosome arm in which knobs do not exist in this population of 365 plants. This portion of each chromosome will be considered as the knobless region.

The study of the relationship of knob position to knob frequency would have been much simpler had all chromosome arms been the same length. The long arms of chromosomes II, IV, VI, and VIII and the short arm of chromosome I, however, are approximately the same length and so, with some propriety, may be averaged. On these 5 arms there are 8 of the 18 known knobs, and these were used to make a preliminary test of the relationship between their frequencies and the positions they occupy on the chromosome arms.

These eight knobs are at positions 15.53μ , 24.17μ , 24.35μ , 25.96μ , 26.43μ , 27.18μ , 29.41μ , and 29.98μ from the fiber attachment (table 4). Their respective frequencies are 13.9, 45.3, 58.8, \longleftrightarrow , 62.7, 57.9, 60.5, 39.4, and 23.5 percent (table 5). The increasing and decreasing array in knob frequency may be interpreted as a function of the distance from the fiber attachment. The frequency increases with distance until a point between 24.35μ and 25.96μ from the fiber attachment is reached; but from this point on, as the distance increases there is a decline in knob frequency. The point of highest knob frequency for arms approximately 36μ long is 25.96μ from the fiber

attachment. It is assumed that a knob located somewhere between 24.35μ and 25.96μ from the fiber attachment would occur with even higher frequency. Consequently, 25.2μ from the fiber attachment is taken as the knob position of maximum frequency. Knobs either side of this position will be found less frequently.

On the assumption that there is a real relationship between position and frequency of occurrence of knobs in the 5 chromosome arms of equal length, a similar relationship is to be expected for the 10 knobs on the remaining 15 arms.

Given the position of 25.2μ from the fiber attachment as the most favorable knob location on the five arms that are approximately 36μ long, the problem becomes one of determining where the corresponding position would be on longer or shorter arms.

The short arms of chromosomes X, VI, VII, and VIII (table 3) seem too short to reach a point at which knobs occur frequently. The next longer arm is the short arm of chromosome IX, which is 15.21μ long. This arm is terminated by a knob (table 4) that occurs with a high frequency (table 5), indicating that this point is close to the most favorable knob position on an arm of this length. Because a knob might occur with even a higher frequency than this terminal knob on the short arm of chromosome IX, it is assumed that a point will form a knob with maximum frequency at 15.2μ from the fiber attachment, a position very close to the end of this arm.

It has been shown above that arms longer by 20μ or more than the short arm of chromosome IX have their most favorable knob positions at approximately 25.2μ from the fiber attachment, a point 10μ farther from the fiber attachment than the terminal knob of chromosome IX. It follows that the most favorable knob position is farther removed from the fiber attachment for long than for short chromosome arms.

Many attempts were made to find a method for determining the expected location of the most favorable knob position with unit increase in arm length above 15.2μ . These attempts were governed by the two points already suggested, the one 15.2μ from the fiber attachment for a chromosome arm 15.21μ long, and the other 25.2μ from the fiber attachment for a chromosome arm approximately 36μ long. The increase of about 21μ in arm length above 15.2μ apparently has moved the position of maximum knob frequency only 10μ farther from the fiber attachment. The relationship between the increase in arm length above 15.2μ and the shift in position seems to be not a straight line but one that increases with each unit increase in length.

The following method was used to find the theoretical position of maximum knob frequency for each arm: Determine the number of microns each arm exceeds 15.2μ in length, square this excess, multiply it by 0.0225, and add the product to 15.2. This gives the position on each arm at which, if a knob-forming point exists, it will form a knob with greatest frequency.

The theoretical position on each of the 20 chromosome arms for maximum knob frequency has been determined by the above procedure and is given in table 6.

Figure 3 shows two positions, one on each arm of chromosome II. Figure 4 shows the curve (broken) that cuts each chromosome arm at the most favorable knob position. This curve does not touch the calculated position for the point on any of the 20 arms, though it does pass through one of the knobs, which of course is many times the

size of a point. In the following paragraphs it will be shown that the frequency of each knob is related to the distance of the knob from the point of maximum frequency as marked by this curve.

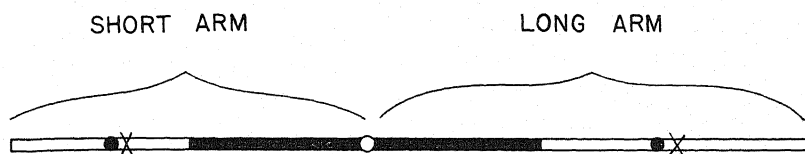


FIGURE 3.—Diagram of chromosome II, showing fiber attachment (circle), arms, knobless regions (solid black), favorable positions for knob-forming points (X), and knobs (solid black circles). $\times 1,500$.

TABLE 6.—Distance on each chromosome arm of most favorable knob position from fiber attachment

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
X.....	Short	μ 14.38	μ 15.05	μ 14.55	II.....	Short	μ 19.61	μ 20.35	μ 19.81
VIII.....	do.....	14.66	15.45	14.85	V.....	Long.....	22.11	21.31	21.89
VI.....	do.....	14.75	15.58	14.96	VII.....	do.....	23.27	23.86	23.45
VII.....	do.....	14.86	15.54	15.03	I.....	Short.....	25.07	24.21	24.82
IX.....	do.....	15.00	15.83	15.20	VIII.....	Long.....	25.03	25.41	25.14
III.....	do.....	15.67	16.36	15.83	IV.....	do.....	24.95	25.87	25.23
IV.....	do.....	16.33	16.67	16.39	VI.....	do.....	26.55	23.86	25.70
X.....	Long.....	18.41	18.47	18.39	II.....	do.....	25.89	25.81	25.87
V.....	Short.....	18.41	18.95	18.53	III.....	do.....	30.41	31.73	30.75
IX.....	Long.....	18.68	19.60	18.91	I.....	do.....	37.59	36.44	37.28

Earlier it was stated that the short arms of chromosomes VI, VII, VIII, and X seemed too short to bear a knob of frequent occurrence. The longest of these four arms has a terminal knob that develops very rarely. This suggested that there is an area adjacent to the fiber attachment that is knobless. The longest of these four short arms extends to a point just slightly beyond this knobless area. This suggestion of a knobless area adjacent to the fiber attachment is supported by the fact that on no chromosome arm is the mean knob position nearer the fiber attachment than 12.44μ .

In determining whether the position of a knob on a chromosome arm affects its frequency, it seemed best to eliminate the knobless region adjacent to the fiber attachment from each arm.

To determine the length of the knobless region adjacent to the fiber attachment for each arm, the knob positions of two of the infrequently occurring knobs, one on a short arm and the other on a long arm, were used as a base. The knob terminating the short arm of chromosome VII has a frequency of 0.6 percent and is 2.59μ from the point calculated as that most favorable for knob formation. If moving the knob 2.59μ from the most favorable position decreases its frequency 99.4 percent, then its frequency should be zero at 2.60μ from the most favorable position, 12.43μ from the fiber attachment. In a similar manner, using the position and frequency of the knob nearest the fiber attachment on the long arm of chromosome VI, it is found that no knob would form at 13.89μ from the fiber attachment.

These two determinations suggested that the length of the knobless area adjacent to the fiber attachment increases with an increase in the length of the chromosome arm.

A method similar to that used in finding the most favorable knob position was used to find for each chromosome arm the length of the knobless region adjacent to the fiber attachment.

The data suggest that an arm shorter than 12.43μ would never bear a knob. The length of the knobless region for each arm was found by determining the number of microns each arm exceeds 12.43μ , squaring

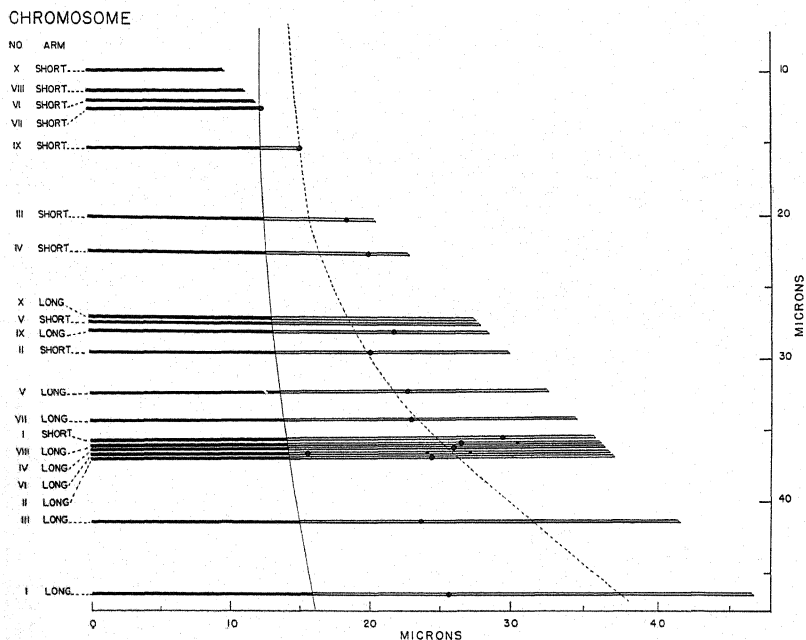


FIGURE 4.—Diagram of the 20 chromosome arms, showing the curve that cuts each arm at the distal end of the knobless region, the curve (broken) that cuts each arm at the most favorable position for a knob-forming point, and the knobs (solid black circles).

this excess, multiplying it by 0.00246, and adding the product to 12.43. By this method the length of the knobless region adjacent to the fiber attachment was found for each arm.

These lengths are given in table 7; figure 3 shows the knobless region adjacent to the fiber attachment for chromosome II; and figure 4 gives the curve that cuts each chromosome arm at the point that terminates the knobless region adjacent to the fiber attachment.

Fixing two points for each chromosome arm gives a piece of definite length on each. This piece lies between the distal end of the knobless region adjacent to the fiber attachment and the theoretical position on the thread of maximum knob frequency. Along this piece knobs will occur with a range of frequencies, depending on their position, from zero to a maximum, the latter for convenience being considered 100. The length of this portion of each chromosome arm is given in table 8.

TABLE 7.—*Length of the knobless part of each chromosome arm adjacent to fiber attachment*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
X-----	Short----	μ 12.50	μ 12.22	μ 12.41	II-----	Short----	μ 13.20	μ 12.99	μ 13.14
VIII-----	do-----	12.51	12.22	12.43	V-----	Long-----	13.53	13.13	13.41
VI-----	do-----	12.51	12.22	12.43	VII-----	do-----	13.66	13.46	13.62
VII-----	do-----	12.51	12.22	12.43	I-----	Short----	13.89	13.51	13.77
IX-----	do-----	12.52	12.25	12.45	VIII-----	Long-----	13.89	13.67	13.82
III-----	do-----	12.66	12.39	12.58	IV-----	do-----	13.89	13.72	13.84
IV-----	do-----	12.75	12.49	12.68	VI-----	do-----	14.06	13.46	13.89
X-----	Long-----	13.06	12.73	12.96	II-----	do-----	13.98	13.72	13.92
V-----	Short----	13.06	12.80	12.98	III-----	do-----	14.53	14.47	14.51
IX-----	Long-----	13.08	12.90	13.03	I-----	do-----	15.39	15.05	15.28

This piece and an equal piece on the distal side of the maximum point make up the knob-bearing region of each chromosome arm. Figure 3 shows diagrammatically chromosome II and its fiber attachment. On each of its arms is shown the knobless region adjacent to the fiber attachment, the position theoretically most favorable for knob development, and the position of its knob.

TABLE 8.—*Distance on each chromosome arm from end of knobless region to most favorable knob position*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
X-----	Short----	μ 1.88	μ 2.83	μ 2.14	II-----	Short----	μ 6.41	μ 7.36	μ 6.67
VIII-----	do-----	2.15	3.23	2.42	V-----	Long-----	8.58	8.18	8.48
VI-----	do-----	2.24	3.36	2.53	VII-----	do-----	9.61	10.40	9.83
VII-----	do-----	2.35	3.32	2.60	I-----	Short----	11.18	10.70	11.05
IX-----	do-----	2.48	3.58	2.75	VIII-----	Long-----	11.14	11.74	11.32
III-----	do-----	3.01	3.97	3.25	IV-----	do-----	11.06	12.15	11.39
IV-----	do-----	3.58	4.22	3.71	VI-----	do-----	12.49	10.40	11.81
X-----	Long-----	5.35	5.74	5.43	II-----	do-----	11.91	12.09	11.95
V-----	Short----	5.35	6.15	5.55	III-----	do-----	15.88	17.26	16.24
IX-----	Long-----	5.60	6.70	5.88	I-----	do-----	22.20	21.39	22.00

Referring to table 4, which shows the position of all knobs, it is possible to determine the distance of each knob from either the 0 or the 100 knob-frequency point. Table 9 gives the distance in microns of each knob from what is considered to be the most favorable knob position, namely, the 100 frequency point. Table 8 shows how many microns of chromosome length on each chromosome produce 100 points of change in knob frequency. From the figures of these two tables it is a simple problem to determine for each knob, from the position it occupies in respect to the 100 point, the reduction in frequency below 100. In table 10 these calculated reductions, converted into percentages, are given for all knobs. In table 11 is given the calculated frequency for each knob, derived by subtracting the reductions of table 10 from 100.

TABLE 9.—*Distance of each knob from most favorable knob position*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
VII.....	Short..	μ 2.34	μ 3.31	μ 2.59	VIIIa ¹	Long....	μ 5.73	μ 4.40	μ 4.84
IX.....do....	.01	.02	.01	VIIIb ²do....	1.63	.57	1.29
III.....do....	2.54	2.84	2.68	IV.....do....	.75	.73	.73
IV.....do....	3.36	3.11	3.62	VIa ¹do....	1.22	1.79	1.48
IX.....	Long....	3.47	1.51	2.81	VIb ²do....	1.66	1.51	1.53
II.....	Short..	.95	1.51	1.18	VIc ³do....	10.24	9.99	10.17
V.....	Long....	.84	.57	.81	II.....do....	1.92	.80	1.52
VII.....do....	.44	.53	.48	III.....do....	6.52	7.96	7.10
I.....	Short..	4.84	4.22	4.59	I.....do....	10.78	13.68	11.67

¹ Knob nearest the end.² Knob in second position from the end.³ Knob in third position from the end.TABLE 10.—*Distance of each knob from the most favorable knob position, expressed as percentage of distance from end of knobless part to most favorable knob position on the respective chromosomes*

Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties	Chromosome		United States Indian varieties	Mexican varieties	Combined United States Indian and Mexican varieties
No.	Arm				No.	Arm			
VII.....	Short..	Percent 99.57	Percent 99.70	Percent 99.61	VIIIa ¹	Long....	Percent 51.44	Percent 37.48	Percent 42.76
IX.....do....	.40	.56	.36	VIIIb ²do....	14.63	4.85	11.39
III.....do....	84.39	70.78	82.46	IV.....do....	6.78	6.01	6.41
IV.....do....	93.85	73.70	97.57	VIa ¹do....	9.77	17.21	12.53
IX.....	Long....	61.96	22.54	48.81	VIb ²do....	13.29	14.52	12.95
II.....	Short..	14.82	20.52	17.69	VIc ³do....	81.99	96.06	86.11
V.....	Long....	9.79	6.97	9.55	II.....do....	16.12	6.62	12.72
VII.....do....	4.58	5.10	4.88	III.....do....	41.06	46.12	43.72
I.....	Short..	43.29	39.44	41.54	I.....do....	48.56	63.95	53.05

¹ Knob nearest the end.² Knob in second position from the end.³ Knob in third position from the end.

The calculated knob frequencies of table 11 agree with the observed frequencies of table 5. The two sets of frequencies are compared by means of the correlation of rank. The coefficient $\rho=0.959$, from a comparison of the observed frequencies and the calculated frequencies of the combined United States Indian and Mexican varieties, indicates the degree of the relationship between the frequency of a knob and its distance from a theoretical position most favorable for knob formation or less directly to its distance from the fiber attachment.

The foregoing discussion of the knob-bearing region of each corn chromosome considers only the data of the combined United States Indian and Mexican varieties. The Indian varieties and the Mexican varieties, however, were treated independently in a manner similar to that for the combined data, and these results also are given, in tables 6 to 11.

TABLE 11.—*Observed and calculated percentage frequency and order of frequency of each knob*

Knob location on chromosome—		Observed		Calculated					
		Fre- quency	Order of fre- quency	United States Indian varieties		Mexican varieties		Combined United States Indian and Mexican varieties	
				Fre- quency	Order of fre- quency	Fre- quency	Order of fre- quency	Fre- quency	Order of fre- quency
No.	Arm	Percent		Percent		Percent		Percent	
IX	Short	89.3	1	99.6	1	99.4	1	99.6	1
V	Long	68.8	2	90.2	5	93.0	6	90.5	4
VII	do	63.2	3	95.4	2	94.9	3	95.1	2
IV	do	62.7	4	93.2	3	94.0	4	93.6	3
VIa ¹	do	60.5	5	90.2	4	82.8	8	87.5	6
II	do	58.8	6	83.9	9	93.4	5	87.3	7
VIIIb ²	do	57.9	7	85.4	7	95.1	2	88.6	5
III	do	54.0	8	58.9	10	53.9	13	56.3	12
VIb ²	do	45.3	9	86.7	6	85.5	7	87.0	8
II	Short	44.9	10	85.2	8	79.5	9	82.3	9
I	do	39.4	11	56.7	11	60.6	12	58.5	10
I	Long	24.5	12	51.4	12	36.0	14	46.9	14
VIIIa ¹	do	23.5	13	48.6	13	62.5	11	57.2	11
IX	do	22.2	14	38.0	14	77.5	10	51.2	13
III	Short	14.7	15	15.6	16	29.2	15	17.5	15
VIc ³	Long	13.9	16	18.0	15	3.9	17	13.9	16
IV	Short	7.4	17	6.1	17	26.3	16	2.4	17
VII	do	.6	18	.4	13	.3	18	.4	18

¹ Knob nearest the end.² Knob in second position from the end.³ Knob in third position from the end.

DISCUSSION AND CONCLUSIONS

The nature and function of the bodies on corn chromosomes, known as knobs, are, as yet, an unsolved problem. The 18 known chromosome knobs have been shown diagrammatically in their mean positions in figures 1, 2, and 4. It is hardly necessary to point out again that these knobs are not distributed uniformly over the whole length of the chromosomes but are restricted in their distribution.

The absence of knobs on those portions of the chromosome threads adjacent to the fiber attachment made it possible to mark off on each chromosome arm a knobless region. The knobless regions are given definite limits in figures 3 and 4, but these may change as the studies of corn chromosomes progress. Two or three years ago several of the infrequently occurring knobs were unknown. At that time the knobless regions would have been longer than those given here.

The knob-bearing region of each chromosome arm increases as the knobless region decreases. The knob-bearing portion of each arm given in the preceding paragraphs fits only the present-day knowledge of chromosome knobs, and as soon as new knobs are found it will be extended, since it is considered that on the chromosomes of any plant 18 is the maximum number of knobs that will occur on the 20 knob-bearing regions herein described.

It is not unusual to find in some plants knobs on the chromosomes that are only slightly larger than the adjacent chromomeres, while in others they are prominent swellings on the thread. This variation in knob size led to the view that there exist 18 points on the chromosomes with the ability to organize knobs and that these points are constant in their position and are always present on the chromosomes of all

corn plants but are visible only when they become knobs. That other knob-forming points may exist outside the present defined knob-bearing areas will not be considered at this time, since this discussion is concerned primarily with the knob frequency at each of the 18 knob-forming points.

The data presented show that knobs on the knob-bearing regions do not occur with equal frequency and that the knob frequency increases as the distance of the knob-forming point from the end of the knobless region (or less directly from the fiber attachment) increases, until a certain position on the thread is reached beyond which the frequency of a knob at a knob-forming point decreases. This certain position on each chromosome arm is the position at which, if a knob-forming point exists, it will form a knob with greater frequency than a point located either nearer to or farther from the fiber attachment.

The knobless or the knob-bearing regions of the chromosomes may be found to change as studies advance, but the position where the knob-forming point forms a knob most frequently is thought to be at a fixed distance from the fiber attachment for each chromosome arm.

Knob formation depends primarily on the presence of a knob-forming point. At present 18 such points are known, all of which are thought to be present on the chromosomes of all corn plants. McClintock⁴ describes a behavior of the nucleolus somewhat analogous to that described above where knob-forming points are shown to exist even when no knob is formed. McClintock found that nucleolar material at a certain stage is associated with all chromosomes but that later this material is drawn to a major organizing center or centers. In the case of knob-forming points, although no single point possesses a knob-forming power that overshadows all other points, there is a marked difference in the different points in their ability to form knobs.

It has been assumed that even the knob-forming point terminating the short arm of chromosome IX is slightly removed from the maximum knob-forming position on this arm, since its frequency is only 89.3 percent. None of the remaining 17 knob-forming points approaches this highest frequency; consequently there seems to be no case in which a knob-forming point coincides with a maximum position.

The differences in knob frequencies (table 5) show that knob-forming points are not alike in their ability to form knobs. It has been the purpose of this paper to show that the differences in knob frequency are related to a difference in position on the chromosome threads of each knob-forming point. The agreement between the observed knob frequencies and frequencies determined by position only has led to the conclusion that knob frequencies and knob positions are correlated.

The correlation between knob position and knob frequency is most readily apparent in chromosome arms of the same length. Most arms, however, are quite unequal in length. Consequently, a method had to be found for determining for chromosome arms of different lengths the position of two points, one having a zero and the other a maximum knob-forming ability. That these points are greater in linear distance from the fiber attachment when a chromosome arm

⁴ MCCLINTOCK, BARBARA. THE RELATION OF A PARTICULAR CHROMOSOMAL ELEMENT TO THE DEVELOPMENT OF THE NUCLEOLI IN ZEA MAYS. *Ztschr. f. Zellforsch. u. Mikros. Anat.* 21: [294]—328, illus. 1934.

is long than when it is short is analogous to the behavior of two points, each an inch from the ends of a 3-inch rubber strap made to stretch unequally by having it one-fourth of an inch across at the base and tapering to one-sixteenth of an inch across at the top. If the base is held firm and the strap stretched from the top, each unit increase in length will change the relative positions of the two points either to the base or to each other. This change is similar to the change in position of the two points on the chromosome arm, one at the distal end of the knobless region and the other at the maximum knob-forming position, with each unit increase in length.

Since the data have shown that the ability of a knob-forming point to form a knob is related to its distance from the fiber attachment, it follows that each chromosome arm possesses a gradient. Darlington and La Cour⁵ have shown something akin to this gradient in their statement that differential staining reaction " * * * seems to depend on distance from the centromere."

It is assumed that there is a gradient along each chromosome arm, which is equal in all at the fiber attachment and which increases toward the end, as measured by knob formation, until a point is reached where a knob-forming point will form a knob most frequently, after which it decreases. With a gradient of this sort it is possible to find a theoretical point on each of the 20 arms having equal power to form a knob, provided there exists at this position a knob-forming point.

It seems reasonable to conclude, from the data thus far accumulated, that no two knob-bearing points are at positions having equal power to form knobs and that the slight differences in position of the knob-forming points, when translated into a difference in gradient along the chromosome thread, account for the observed differences in knob frequency.

Since knobs are found also on the chromosomes of the close relatives of corn, it seemed likely that data on the frequency and position of knobs from these relatives would throw additional light on this problem of knob formation. A limited study of the Florida and Moyuta strains of annual teosinte (*Euchlaena mexicana* Schrad.) has provided only sufficient data to give an indication of the relationship between knob frequency and knob position in this related genus. These meager data show that the five shortest arms and the two longest arms are without knobs and that knobs at the end of arms having a certain length are present more frequently than are knobs at the end of arms, both longer and shorter. The maximum point for knob formation seems further removed from the fiber attachment than is the case on the arms of corn chromosomes.

In conclusion, if the ability of knob-forming points to collect knob material is controlled by a differential gradient along the chromosome thread, it seems reasonable to infer that other features of the chromosome are affected by this gradient.

The foregoing discussion goes no further than to propose that knob frequency is related to the position of a knob-forming point on the chromosome thread.

Position of a knob-forming point may not be the only factor controlling knob formation. An examination of the knobs on the chromo-

⁵ DARLINGTON, C. D., and LA COUR, L. DIFFERENTIAL REACTIVITY OF THE CHROMOSOMES. *Ann. Bot. [London]* (n. s.) 2: 615-625, illus. 1938.

somes of corn from different regions clearly shows that the amount of knob material is not the same in all plants. Consequently, the 18 knob-forming points thought to be present on the chromosomes of all corn plants may not have, in different plants, the same amount of material for knob formation.

Assuming that the quantity of material available interacts with the position of knob-forming points to affect the frequency of knob formation, the following points are suggested for consideration in future studies:

- (1) The amount of knob material varies from plant to plant.
- (2) The number of knobs on the chromosomes of a plant is limited by the amount of material available. This material is usually insufficient to fill all knob-forming points.
- (3) The position of a knob-forming point determines its ability to collect from the available knob material.

Consequently, the knob-forming point nearest a point of maximum knob-forming power will be filled with knob material most frequently and the point farthest from a point of maximum knob-forming power will be filled with knob material least frequently.

SUMMARY

A study of the midprophase of the first meiotic division of pollen mother cells from 74 varieties of corn of the United States and Mexico has shown that knobs are not distributed uniformly over the whole length of the chromosome but are at points on the thread at an appreciable distance from the fiber attachment.

The frequency of a knob has been shown to be a function of the distance of a knob-forming point from the fiber attachment.

The effect of the fiber attachment upon knob frequency is shown by the failure to find knobs in close proximity to the fiber attachment. The depressing effect of the fiber attachment on knob formation decreases as the distance from the attachment region increases, until a point is reached on each arm where knob formation is at a maximum. This change in knob-building power is interpreted to mean that each chromosome arm possesses a gradient.

A preliminary study of the Florida and Moyuta strains of annual teosinte reveals a relationship between the frequency of the knobs and the distance of the knob-forming points from the fiber attachment similar to that outlined for corn.

CATION EXCHANGE PROPERTIES OF CERTAIN FOREST SOILS IN THE ADIRONDACK SECTION¹

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INTRODUCTION

The central Adirondack section in New York State offers an excellent opportunity for studying the properties of virgin forest soils. There are localities where the climax forest types have not been appreciably disturbed by man or fire. It is the purpose of this paper to present data on the cation-exchange capacity, exchangeable bases, percentage base saturation, percentage loss on ignition, and hydrogen-ion concentration for soils of similar geological origin but occurring beneath three distinct forest types, namely, red spruce, red spruce-sugar maple-beech, and sugar maple-beech-yellow birch. Furthermore, some general relationships are shown between organic-matter content, percentage base saturation, pH values, and the apparent pK values² of the soils.

DESCRIPTION OF FOREST TYPES

The study was conducted in the general vicinity of Newcomb, N. Y. The climate and geology of this locality have been adequately described elsewhere (1, 7, 17).³

The three forest types selected are briefly described as follows:

(1) Red spruce type. This is type 18 described in the report of the committee on forest types of the Society of American Foresters (16). It also corresponds to the *Oxalis-Cornus* type described by Heimbürger (7, pp. 32-33). The type is composed chiefly of red spruce, with scattered specimens of yellow birch, balsam fir, red maple, beech, and hemlock.

(2) Red spruce-sugar maple beech type. This is type 17 in the Society of American Foresters' classification (17). It corresponds to the *Viburnum-Oxalis* type of Heimbürger (7, pp. 33-34). It is composed chiefly of red spruce, sugar maple, yellow birch, beech, red maple, and scattered balsam fir and hemlock. This is the most common forest type in the central Adirondacks.

(3) Sugar maple-beech-yellow birch type. This is type 12 in the Society of American Foresters' classification (16) and more specifically corresponds to the *Arisaema* type of Heimbürger (7, pp. 36-37). The general northern hardwoods type varies considerably in species composition. The type used here represents that on the richer sites in the Adirondack section. It is composed of sugar maple, beech, and yellow birch in varying proportions, with smaller admixtures of basswood, white ash, black cherry, and hophornbeam.

¹ Received for publication April 25, 1939.

² The apparent pK value is the logarithmic reciprocal of the dissociation constant of the soil acids. The method for its estimation is presented later.

³ Italic numbers in parentheses refer to Literature Cited, p. 504.

For convenience, in this paper, these three types will be referred to, in the order described, as the spruce type, spruce-hardwood type, and hardwood type.

The stands selected for study with respect to the influence of forest type upon exchangeable cations were essentially mature, virgin forests, with the exception that there had been a selective cutting of spruce and balsam on the spruce-hardwood type, thus decreasing the percentage of those species in the stands.

DESCRIPTION OF SOIL TYPES

In selecting the locations for obtaining soil samples for this study, the principal criterion was geological origin. The soils selected consisted of glacial till soils of similar texture in which Adirondack gneiss was the predominating rock. The profiles beneath the spruce and spruce-hardwood types were those of mature Podzol soils. The profiles beneath the hardwood types represented the Brown Podzolic soils. The humus layers⁴ varied beneath the different forest types, there being a greasy mor under the spruce stand, a granular mor under the spruce-hardwood stands, and a fine mull beneath the hardwood stands. The essential morphological features of the profiles are presented in table 1.

TABLE 1.—Description of glacial till soils used in this study

Horizon	Spruce stand	Spruce-hardwood stand	Hardwood stand
A ₀ -----	Greasy mor humus layer about 8 inches thick with a black amorphous H layer ¹ predominating.	Granular mor humus layer about 6 inches thick with black granular H layer predominating.	L and F layers about 1 inch thick H layer essentially absent. Fine mull humus layer.
A ₂ (A ₁ under hardwoods).	Gray, leached loamy sand, 3 inches thick.	Gray, leached loamy sand 3 inches thick.	Mixture of organic matter and mineral soil. Organic matter content over 30 percent. About 3 inches thick.
B ₁ -----	Very dark brown sandy loam. Relatively high in organic matter. Average thickness about 2 inches.	Dark coffee-brown sandy loam. High in organic matter, about 3 inches thick.	Dark chocolate brown loam, about 8 inches thick.
B ₂ -----	Rusty-brown sandy loam, about 5 inches thick.	Rusty-brown sandy loam, about 5 inches thick.	Yellowish-brown sandy loam about 10 inches thick.
B ₃ -----	Yellowish-brown sandy loam, about 7 inches thick.	Yellowish-brown sandy loam, about 8 inches thick.	Yellowish-gray sandy loam, about 9 inches thick.
C ₁ -----	Greenish-yellowish gray loamy sand. Very compact.	Greenish-yellowish gray loamy sand. Very compact.	Greenish-yellowish-gray loamy sand. Very compact.

¹ The terms, L, F, and H layer as used in this paper may be defined as follows: L layer is composed of freshly fallen leaves that have not appreciably decomposed; F layer is composed of litter in process of decomposition but which is still recognizable as to source; H layer is composed of litter that has decomposed to such an extent as to not be recognizable as to source. H is usually a black, finely divided, amorphous layer.

Two profiles were sampled beneath the spruce-hardwood and hardwood stands, but data were secured from only one profile beneath the spruce stand. The description is the average of all profiles beneath a given forest type. All sampling areas were within a radius of 10 miles of Newcomb. An inspection of table 1 reveals that the profiles beneath the spruce and spruce-hardwood stands

⁴ Classification of Bornebusch and Heiberg (3).

were very similar, the principal difference being in the humus layer. The profiles beneath the hardwood stands were markedly different, being rich fine mulls, with an intense incorporation of organic matter in the A₁ horizon. The organic matter penetrated well into the B horizon and gave the soils a chocolate-brown color and a fine granular structure.

The soils beneath a single forest type varied very little from one locality to another. Two samples from a given forest type seemed adequate to characterize the soil beneath it. In fact, the correlation between vegetation and soils was so high that one could predict quite accurately the general type of profile from observation of the forest type and its characteristic ground vegetation.

The soil types have not been mapped in the Adirondack section, but the soils beneath the spruce, and the spruce-hardwood stands seemed to be closely related to the Beckett sandy loam type, while those beneath the hardwood stands corresponded to descriptions of Essex sandy loam type. Hence, two great soil groups were represented the Podzol and the Brown Podzolic.

In addition to the soils described above, some special studies were carried out on two other soils which were in the same general section, but which were coarser textured and were derived from glacial outwash. These soils corresponded quite well with those of the Colton series. They were mature humus Podzols and occurred beneath stands of the red spruce type.

In all cases a large soil pit was dug, so that the soil profile could be carefully examined. The samples secured were composites of soil taken from the sides of the pits.⁵ The soils were air-dried and sifted through a 2-mm. sieve before analysis. All determinations were made on air-dry soil.

LABORATORY METHODS

The selection of methods for the determination of exchangeable cations in forest soils requires special attention because of the high content of organic matter in certain horizons. The cation, as well as the anion in the leaching salt, markedly affects the solubility of the organic matter. The ammonium ion is particularly active in this respect, and hence methods should be avoided which involve the treatment of soils with any ammonium salt previous to the determination of total exchange capacity. In order to demonstrate the effect of ammonium acetate upon the reduction of the exchange capacity of forest soil samples, two organic layers and two C-horizon samples were treated in two different ways. One set was treated with neutral normal ammonium acetate solution, the exchangeable ammonium was replaced with calcium and the amount of exchangeable calcium determined to give exchange capacity. The other set was treated with 0.05 N hydrochloric acid and barium acetate solution in accordance with the procedure described later. The results are reported in table 2.

⁵ The author is indebted to R. L. Donahue and H. T. Hopkins for obtaining certain of the samples.

TABLE 2.—Comparison of ammonium acetate and hydrochloric acid treatments in determining exchange capacity of forest soils

Organic matter (percent) in H layer and C horizon	Hydrochloric acid barium acetate treatment			Ammonium acetate—calcium acetate treatment		
	Exchange capacity per 100 gm. ¹	Exchangeable hydrogen per 100 gm.	Base saturation	Exchange capacity per 100 gm.	Exchangeable hydrogen per 100 gm.	Base saturation
	Milli-equivalents	Milli-equivalents	Percent	Milli-equivalents	Milli-equivalents	Percent
H layer, 88.6	148.2	128.7	13.1	130.6	127.8	2.1
H-layer, 81.8	158.0	135.7	14.1	129.0	134.5	-----
C-horizon, 0.93	2.6	2.0	23.1	2.6	2.0	23.1
C-horizon, 4.35	6.4	5.2	18.7	6.3	5.3	16.0

¹ Dry soil.

There was a decided reduction in exchange capacity of the H-layers when treated with ammonium acetate. The C-horizons, being low in organic matter, were not appreciably affected. An effect similar to this would explain why Lunt (8) obtained higher values for exchangeable hydrogen than for exchange capacity in the organic layers of New England forest soils. It might also explain why Lutz (9) obtained percentage-base-saturation values of less than 10 for certain A₀ horizons with a pH value greater than 4.5.

In this study the hydrochloric acid-barium acetate method was used for determining the value for exchange capacity and exchangeable hydrogen. Two portions of a given sample were weighed out. One portion was used for the determination of the exchange capacity, while the other was used in the determination of exchangeable hydrogen. The exchange capacity was determined as follows: A sample of soil (1 to 10 gm. depending on the exchange capacity of the material) was weighed out and transferred to a 150-ml. beaker. About 50 ml. of 0.05 N hydrochloric acid solution was added. The mixture was allowed to stand for several hours, with occasional stirring. Then the sample was filtered on asbestos, by using suction, and washed with successive 25-ml. portions of acid until 300 ml. had passed through the filter. The soil was then washed with water until the leachate was free from chlorides. The soil and asbestos were transferred to the beaker, and 50 ml. of neutral normal barium acetate was added. After several hours the mixture was again filtered and washed with 400 ml. of barium acetate, and the acetic acid in the leachate was titrated potentiometrically with 0.1 N barium hydroxide.

Exchangeable hydrogen was estimated by treating the soil directly with neutral normal barium acetate solution, and titrating the acetic acid as described above. The amounts of exchangeable bases were determined by obtaining the difference between exchange capacity and exchangeable hydrogen.

This procedure has the advantage of not dispersing the organic matter, and of permitting the titration of exchange capacity and exchangeable hydrogen to the same end point. The method is relatively rapid, as the final determinations are volumetric. Also, when either barium or calcium acetate are used, one cannot determine exchangeable calcium, but it can be determined in the 0.05 N filtrate hydrochloric acid filtrate secured in the determination of exchange capacity.

The pH value was determined potentiometrically in a thick soil-water suspension, a glass electrode being used.

Organic matter was estimated by loss of weight on ignition at a temperature of about 500° C. The values are doubtless slightly high, but since the soils were free from carbonates and contained less than 5 percent of clay, it seemed unnecessary to resort to more time-consuming methods. Hence organic matter is used in this paper as represented by the loss-on-ignition figures.

The pK values reported were calculated from the cation exchange data by the well-known formula:

$$pH = pK + \log \frac{\text{salt}}{\text{acid}}$$

Milliequivalents of bases represented salt and milliequivalents of hydrogen represented acid.

In order to check the accuracy of this procedure, 10 samples were half saturated with calcium hydroxide so as to cause the logarithm of the salt-acid ratio to become zero, and hence the pH value to become equal to the pK value. The results of these determinations were somewhat higher than those calculated directly (5.0 as compared with 4.8), but the correlation between them was high. The calculated values are reported in this paper.

The pK values give somewhat empirical figures for the strength of the soil acids, since the formula for their calculation is designed for use in simple systems involving a single monobasic acid. For this reason the constant is referred to in this paper as "apparent pK value." The results obtained, however, indicate that the constant is of considerable value in characterizing soils. The assumption is made that as the apparent pK value decreases, the dissociation of the soil acids increases. It is used to indicate the importance of the intensity as well as the capacity factor in soil-acidity studies.

EXPERIMENTAL RESULTS

In table 3 are presented the average data for the five profiles. In order to make certain relationships clear, the data are also presented graphically. The vertical scale for horizons assumes all layers to be of the same thickness. Also the graphs were drawn by connecting the points corresponding to the values determined for each horizon. This method of presentation was used to simplify the comparisons. If the actual horizon depths were plotted on the vertical scale and a block graph drawn for each horizon, it would be necessary to plot three graphs for each set of values and comparisons would be somewhat more difficult. An inspection of table 1 indicates that the depths of the B₁ horizons were essentially equal in all profiles; and since the values change gradually with depth, the straight lines probably represent the actual situation for the B and C horizons. No values are reported for the B₂ horizons because preliminary evidence indicated that the figures were always intermediate between those for the B₂ and C horizons and hence would not add any particular value to this report. To further simplify comparisons, the data for the A₁ horizon of the hardwood profile are plotted in the figures as though they applied to the A₂ horizon.

TABLE 3.—*Exchange capacity, exchangeable hydrogen, exchangeable bases, percentage base saturation, pH values apparent pK values, and percentage loss on ignition of soils beneath three forest types*

Forest type and horizon	Exchange capacity per 100 gm. air-dry soil	Exchangeable hydrogen per 100 gm. air-dry soil	Exchangeable bases per 100 gm. air-dry soil	Base saturation	pH	pK ¹	Loss on ignition
	<i>Milli-equivalents</i>	<i>Milli-equivalents</i>	<i>Milli-equivalents</i>	<i>Percent</i>			<i>Percent</i>
Spruce:							
A ₀ (H layer).....	148.2	128.7	19.5	13.1	3.45	4.27	88.6
A ₂	8.0	6.4	1.6	20.0	4.60	5.22	3.98
B ₁	56.0	40.6	15.4	27.5	4.75	5.17	20.6
B ₂	29.3	21.4	7.9	27.0	4.95	5.38	12.0
C ₁	2.6	2.0	.6	23.1	5.05	5.53	.93
Spruce-hardwood:							
A ₀ (H layer).....	120.2	103.2	26.0	20.1	3.74	4.34	73.6
A ₂	5.3	4.5	.8	15.1	4.03	4.75	2.25
B ₁	48.9	38.8	10.1	20.7	4.35	4.93	13.8
B ₂	31.8	22.7	9.1	28.6	4.86	5.25	14.0
C ₁	3.3	2.3	1.0	30.3	5.27	5.63	2.01
Hardwood:							
A ₀ (F layer).....	122.5	33.8	88.7	72.4	5.56	5.15	79.7
A ₁	55.8	29.5	26.3	47.1	5.05	5.10	33.3
B ₁	30.8	19.7	11.1	36.0	5.14	5.39	17.0
B ₂	27.2	17.9	9.3	33.8	5.24	5.52	12.5
C ₁	4.7	3.1	1.6	34.0	5.32	5.60	2.8

¹ pK is the logarithmic reciprocal of the dissociation constant of the soil acids.

Figure 1 presents the data for total exchange capacity of the various horizons of the profiles beneath the three forest stands.

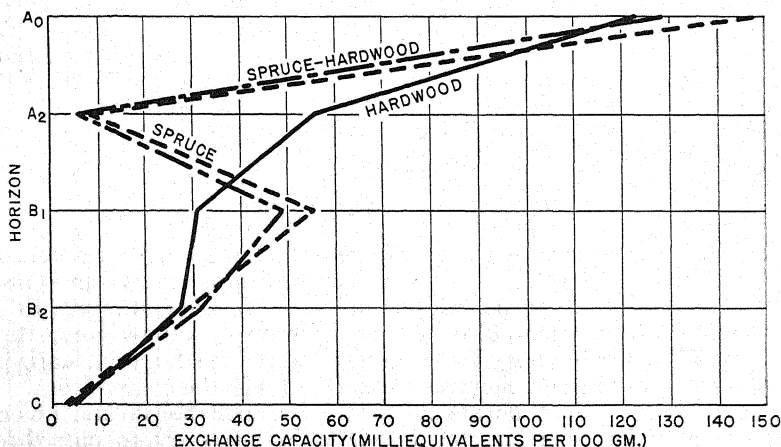


FIGURE 1.—Vertical distribution of total exchange capacity of the horizons of the profiles beneath the three forest types.

The values for the spruce and spruce-hardwood profiles were similar and were typical of humus-Podzol soils in the northeastern part of the United States. The hardwood profiles showed a different distribution, being somewhat higher in the leached horizon and somewhat lower in the B₂ horizon. These values were closely associated with the organic-matter content of the soils. This relationship is shown in figure 2 by plotting percentage loss on ignition against total exchange capacity. The line drawn through the dots was fitted in accordance

with the empirical curve formula for a straight line, $a+bx=y$. After solving for a and b , the formula became $-2.7+0.56x=y$. where x = total exchange capacity and y = percentage loss on ignition. The coefficient of correlation was 0.980. All samples used in this study were plotted individually. Figure 2 shows clearly that the organic matter is the seat of the exchange capacity of these Adirondack forest soils.

In order to indicate the magnitude of the organic exchange complex, the organic matter in the samples from one of the spruce-hardwood profiles was oxidized with hydrogen peroxide in accordance with the method of Olson and Bray (14). The exchange capacity of the

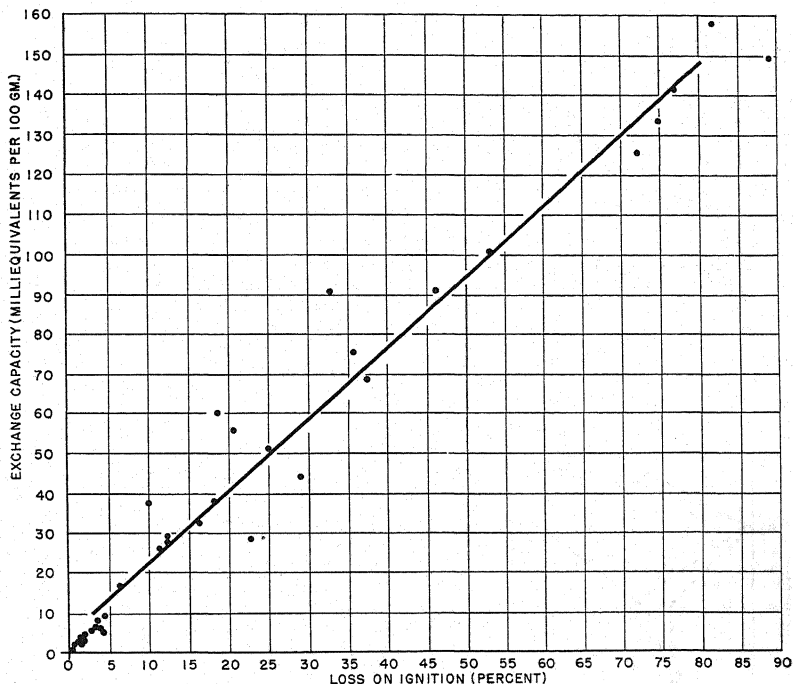


FIGURE 2.—Correlation between percentage loss on ignition and total exchange capacity for soils under hardwood.

H_2O_2 -treated soil was determined. Figure 3 shows the curves for the natural and oxidized soils. This procedure does not accurately evaluate the organic-exchange capacity of the soils, as claimed by Olson and Bray (14), and Bartlett et al. (2). Meyers (12) has demonstrated that organic and inorganic exchange capacities are not additive. If the organic-matter content of a soil is very low, and the inorganic-exchange capacity is high, it is possible to increase the exchange capacity by removing the organic matter. Nevertheless, if one can assume that the exchange capacity of the inorganic fraction was not appreciably altered by the hydrogen peroxide treatment, the figure shows that the exchange capacity of the soil would be very low if the organic matter were not present.

The values for exchangeable bases are presented in figure 4.

Although the graphs show trends similar to those for total exchange capacity, there were certain important differences. The hardwood profile showed a very high concentration of bases in the A_0 and A_1 horizons. In the spruce and spruce-hardwood profiles there was a tendency for a slightly higher concentration of bases in the humus layer of the latter. Although there are only a few figures reported

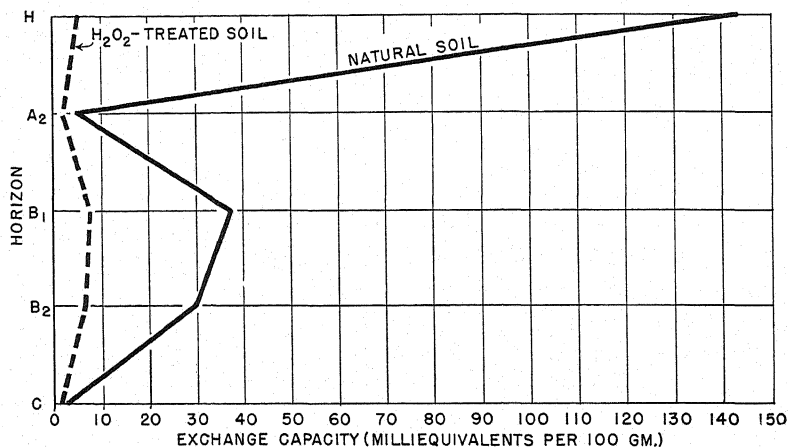


FIGURE 3.—The influence of removal of organic matter upon the exchange capacity of the various horizons of a Podzol profile beneath a spruce-hardwood stand.

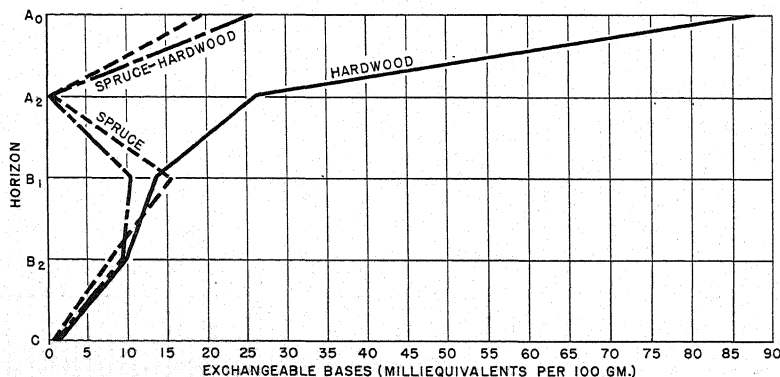


FIGURE 4.—Exchangeable bases in the various horizons of the profiles beneath the three forest types.

here, a large number of pH determinations made on other samples have shown this relationship to be consistent. These differences are most logically explained by the variation in the calcium content of the litter of the trees growing on the various sites. This matter will be treated further in the discussion of results. Because of the variability in exchange capacity among the various horizons, the values for exchangeable bases are better presented in the form of

percentage base saturation. These values, along with those for hydrogen-ion concentration, are plotted in figure 5.

The spruce and spruce-hardwood profiles exhibited similar trends. The hardwood profile showed a higher percentage base saturation as well as pH value in the surface soil although the differences in the B and C horizons were not large. The tendency was for percentage base saturation and pH value to increase with depth beneath the spruce and spruce-hardwood stands, while the opposite tendency

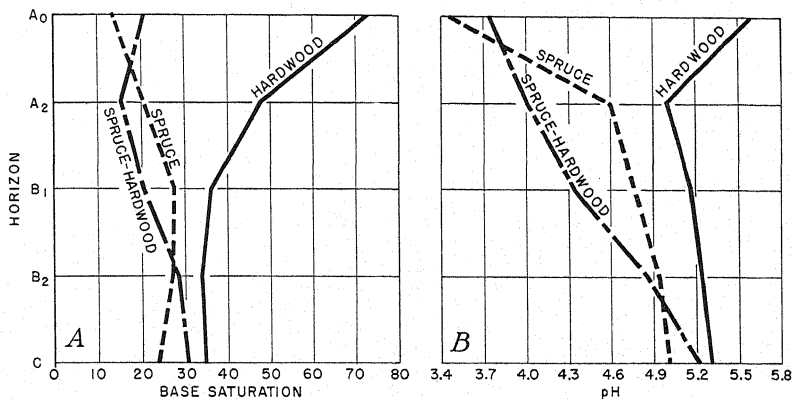


FIGURE 5.—Percentage base saturation (A) and pH values (B) of soils beneath the three forest types.

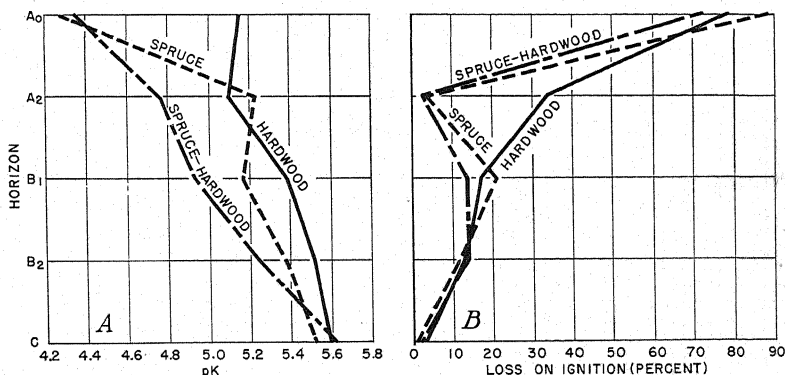


FIGURE 6.—Apparent pK values (A) and loss on ignition (B) for the various horizons of the profiles beneath the three forest types.

prevailed with the hardwood stand. The pH values increased with depth to a greater degree than did the percentage-base-saturation values. This increase was undoubtedly owing to the differences in strength of the soil acids and was associated with the organic-matter content of the soil. This point is supported by figure 6.

As the organic matter decreased, the apparent pK values tended to increase. This was not an exact relationship, and there were some exceptions to the rule. The A₀ horizon of the hardwood plots tended

to have a high apparent pK value, even though the organic-matter content was similar to the other A_0 horizons. This might be due, in part, to the fact that the degree of decomposition of the leaf litter was not so advanced. Furthermore, when the material becomes well decomposed in this type of soil, it is already mixed with the mineral soil. The apparent pK value for the A_2 horizon seemed rather low, in consideration of its organic-matter content. The inorganic-exchange capacity of the A_2 horizon was low, and what organic matter was present might have had a relatively great influence upon the salt-acid ratios at a given pH value. The steady increase in apparent pK value in passing downward from the B_1 horizon correlated well with the organic-matter content.

In certain humous Podzols on light-textured soils, the observation has been made that the pH value of the B_1 horizon was lower than that of the A_2 . Such a profile has been described by Lyon and Buckman (10) and has caused some comment, because one naturally considers the A horizon to be more thoroughly leached than the B horizon and hence that it should be more acid.

Two such profiles from the Adirondack section were sampled. They were both strongly podzolized soils, originating from coarse sandy glacial outwash. Forest stands predominating in red spruce covered the sites. The organic-matter content of the B_1 horizon averaged 28.3 percent, that of the A_2 horizon only 1.69 percent; thus they were pronounced humus Podzols. The samples were analyzed for the same properties as for the profiles beneath the different stands. The results are reported in table 4.

TABLE 4.—Average values for exchange capacity, exchangeable hydrogen, exchangeable bases, percentage base saturation, pH values, apparent pK values, and loss on ignition for two Podzol profiles derived from glacial outwash

Horizon	Exchange capacity per 100 gm	Exchangeable hydrogen per 100 gm	Exchangeable bases per 100 gm	Base saturation	pH	Apparent pK	Loss on ignition
	Milli-equivalents	Milli-equivalents	Milli-equivalents	Percent			Percent
A_0 (H layer)	140.6	114.2	26.4	18.8	3.65	4.38	70.0
A_2	3.35	2.67	.68	20.3	4.56	5.25	1.69
B_1	71.3	53.8	17.5	24.5	4.06	4.55	28.3
B_2	41.9	32.7	9.2	21.9	4.68	5.22	14.9
C	3.7	3.2	.5	13.5	4.61	5.41	2.4

In figure 7 are curves showing the trends for hydrogen-ion concentration, apparent pK values, and percentage base saturation.

Instead of the usual correlation between hydrogen-ion concentration and percentage base saturation, it was found that when the hydrogen-ion concentration increased, the percentage base saturation usually went down, and when they did increase together the increase in hydrogen-ion concentration was correspondingly greater than that for percentage base saturation. The apparent pK values showed the reason for this. The apparent pK value of the A_2 horizon showed a decided increase over that of the A_0 horizon. The pH value also increased about 1 pH unit. The percentage base saturation, however, increased only from 18.8 to 20.3 percent. The apparent pK

value dropped again in the B₁ horizon, and the percentage base saturation actually increased from 20.3 in the A₂ horizon to 24.5 in spite of the reduction in pH value from 4.56 to 4.06. It is the writer's belief that the difference in apparent pK value between the A₂ and B₁ horizons explains the occurrence of a lower hydrogen-ion concentration in the illuvial than in the eluvial horizon. The stronger acids in the B₁ horizon can bind more bases at a lower pH value than can the weaker acids in the A₂ horizon. All the profiles studied have consistently shown a greater degree of base saturation in the B₁ horizon than in the respective A₂ horizon regardless of the pH values. One would expect a lower hydrogen-ion concentration in the B₁ only in profiles which have a high accumulation of organic matter in the B₁ horizon and which have a very low inorganic-exchange capacity.

DISCUSSION OF RESULTS

The high content of exchangeable bases as well as the high percentage base saturation in the surface soil associated with the hardwood

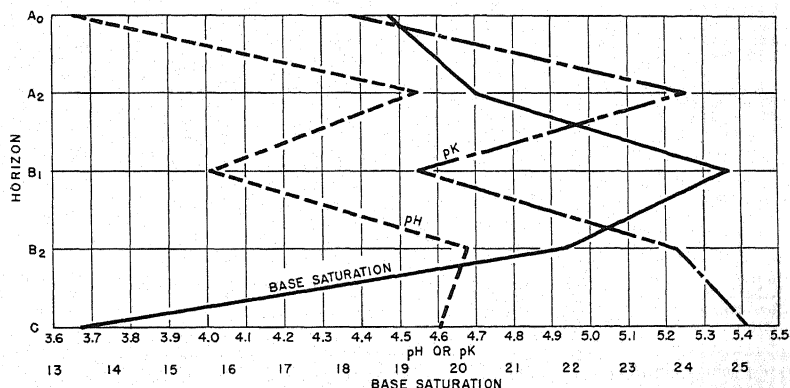


FIGURE 7.—Hydrogen-ion concentration, apparent pK values, and percentage base saturation for the sandy, glacial outwash, Podzol profiles.

stands was the most conspicuous influence of forest type upon the exchangeable cations in the soil. The fact that all the B and C horizons were so similar in this respect leads one to believe that the influence was truly associated with the forest type. It has been shown by several workers (6, 11, 13) that the calcium content of the tree leaves of different species may vary a great deal. That this calcium is important in influencing the pH value of the surface soil has also been demonstrated (4, 5, 15). In order to determine what relationship exists between the calcium content of the foliage of the trees and the exchangeable bases in the soil, leaf samples were secured from these trees in September 1938 and were analyzed for total calcium. Since the crown area of the trees is important in determining the amount of litter deposited and since basal area is closely correlated with crown area, the diameters of all trees in a circular area comprising 0.1 acre immediately surrounding the soil pit were taken and the data were expressed as percentage basal area by species. These

values were multiplied by the percentage calcium content of the foliage of each species, the summation of which gave an empirical figure that represented the relative amount of calcium deposited on a particular area. These data are presented in table 5.

TABLE 5.—*Proportion of the basal area of the 0.1 acre immediately surrounding the soil pit occupied by the different species along with the calcium content of the foliage and the relative amount of calcium added to the soil*

Type and species	Proportion total basal area occupied	Calcium content of foliage	Relative amount of calcium added to the soil ¹	Type and species	Proportion total basal area occupied	Calcium content of foliage	Relative amount of calcium added to the soil ¹
Hardwood:	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	Spruce-hardwood—continued	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Sugar, maple....	41.3	1.36	58.5	White ash.....	0		
Basswood.....	23.2	2.82	65.2				
Beech.....	20.1	.75	15.1	Relative amount of calcium added to soil.....			96.3
Yellow birch.....	8.9	1.34	11.7				
Red spruce.....	0						
Hemlock.....	0			Spruce:			
Balsam fir.....	0			Sugar maple.....	0		
Red maple.....	0			Basswood.....	0		
American elm.....	1.9	2.00	3.8	Beech.....	5.0	.63	3.1
White ash.....	4.6	1.91	8.8	Yellow birch.....	25.4	1.21	27.9
Relative amount of calcium added to soil.....			163.1	Red spruce.....	61.3	.85	52.1
				Hemlock.....	2.1	.71	1.6
Spruce-hardwood:				Balsam fir.....	3.2	.96	3.1
Sugar maple.....	39.9	1.01	40.4	Red maple.....	3.0	.84	2.5
Basswood.....	0			American elm.....	0		
Beech.....	24.0	.60	14.4	White ash.....	0		
Yellow birch.....	27.2	1.27	34.5				
Red spruce.....	2.1	.87	1.8	Relative amount of calcium added to soil.....			90.3
Hemlock.....	6.3	.75	4.7				
Balsam fir.....	.2	.96	.2				
Red maple.....	.3	.95	.3				
American elm.....	0						

¹ Obtained by multiplying the percentage basal area by the figures for calcium content of foliage.

The relative value of 163.1 for the hardwood stand is very much larger than the values of 96.3 and 90.3 for the spruce-hardwood and spruce stands. The low value for the spruce-hardwood stand as compared with the hardwood stand was caused by the absence on the former area of such high-calcium species as basswood, American elm, and white ash. In addition, the trees of the same species tended to absorb considerably more calcium on the hardwood sites. This was especially true with respect to sugar maple. Although exchangeable calcium was not determined on many samples, preliminary results, as well as those obtained by other workers, indicated that exchangeable calcium represented from 75 to 90 percent of the total bases.

These calculations assume, of course, that the trees deposited amounts of litter in proportion to their basal areas and hence can only be considered as rough estimates. But the trend is doubtless well indicated by the figures. The data in table 5 show the fallacy of the common assumption that the foliage of all hardwoods has a high calcium content as compared with that of coniferous trees.

The results obtained in this study tie in with previous studies made in the same section. Hopkins⁶ has recently shown that the concentration of tree roots is highly correlated with the moisture equivalent and percentage loss on ignition of these same soils. Donahue⁷ has demonstrated the greater productivity of the hardwood sites as compared with the spruce-hardwood and spruce areas. Heimbürger (7) has shown that the soils under this hardwood type nitrify much more than do soils under the other two types. It is the writer's opinion that these differences in productivity are caused largely by the influence of the type of litter deposited annually.

The question naturally arises, however, as to why different forest types occur on soils presumably identical at the end of the last glacial epoch. This is a moot question, to which there are several possible answers:

- (1) The distribution of the trees may have been accidental originally, but once established they continued to reproduce themselves and made the site more or less favorable, depending on the species present.

- (2) There may be differences in the soil characteristics which have not been discovered, as yet.

- (3) The water conditions as determined by topography may have been influential in determining the distribution of tree species.

This last point seems to be rather likely, although it has not been proved. Observation of some 20 soil pits in the Adirondacks during the season of 1938, which happened to be an exceptionally wet one, revealed that the pits in the hardwood sites always had a certain amount of moving ground water passing through them, and that during extremely rainy spells a free water table was present on top of the compact C horizon. This situation did not prevail on the spruce-hardwood or spruce sites. At no time was there a perched water table in the soils in these sites. There were no apparent differences in permeability of the parent material. The influence evidently arose from the topographical features of the areas, the hardwood sites always being situated near the base of a long slope, with an opportunity for extra water to move down the hillside. If extra water is available on these sites during wet periods, in all probability the areas would not suffer so seriously during dry periods. This point needs further study. But if such is the case, the difference in species composition might be due to the fact that during periods of drought some of the more exacting species such as basswood, white ash, and even sugar maple were eliminated from the sites where sufficient water did not exist. It should be mentioned here that the spruce-type profile was not a very characteristic one, because the majority of the spruce stands in the Adirondacks occur on either lighter-textured soils and/or soils that are poorly drained and have a high, stagnant water table. Therefore the foregoing discussion applies more particularly to the hardwood and spruce-hardwood types.

⁶ HOPKINS, H. T. ROOT DISTRIBUTION OF TREES IN THE ADIRONDACK REGION. M. S. thesis, Cornell Univ. 1939.

⁷ DONAHUE, R. L. FOREST GROWTH AND SOIL MORPHOLOGY IN THE CENTRAL ADIRONDACK REGION. Ph. D. thesis, Cornell Univ. 1939. (In preparation.)

SUMMARY

On soils of similar geological origin, the sugar maple-beech-yellow birch forest type produced a soil type which, in the surface layers, was less podzolized, had a higher pH value, and a larger percentage base saturation than soil profiles beneath stands of the red spruce—sugar maple—beech type, or the red spruce type. The differences between the latter two types were small.

Evidence is submitted to indicate that the cause of these differences was associated with the calcium content of the foliage of the trees.

A high correlation existed between total exchange capacity and percentage loss on ignition. Percentage base saturation and pH value were highly correlated when soils of similar organic matter content were compared.

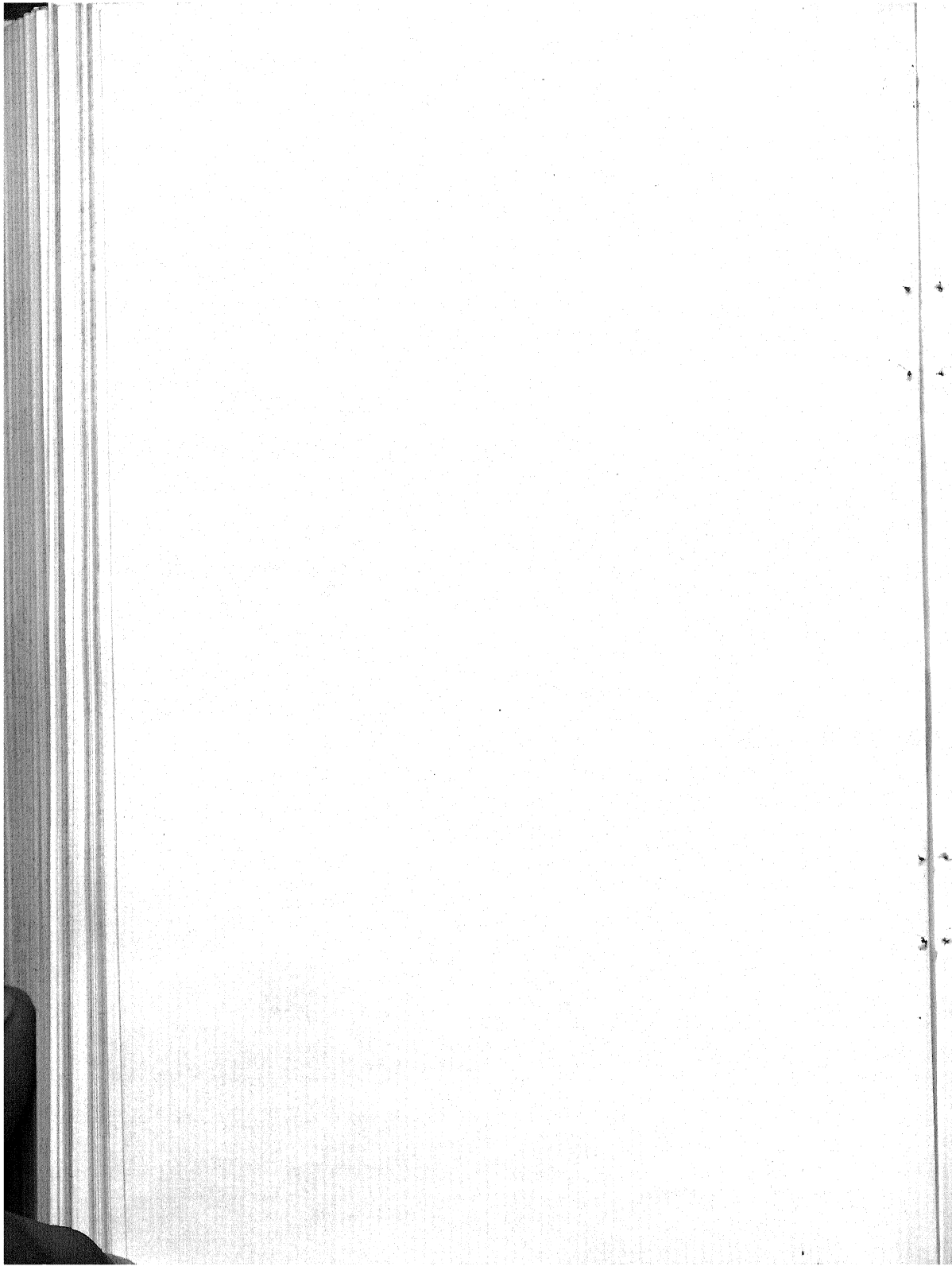
The apparent pK values of the various horizons indicated that the soil organic matter had a lower apparent pK value than that of the mineral soil. The values were somewhat higher for the hardwood profiles, indicating that weaker acids were involved.

The lower apparent pK value of organic matter and hence the higher percentage base saturation at a given hydrogen-ion concentration, explained why it was possible to have a lower pH value in the B₁ horizon than in the A₂ horizon of a humus podzol profile.

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RETENTION OF SOME PHOSPHORUS COMPOUNDS BY SOILS AS SHOWN BY SUBSEQUENT PLANT GROWTH¹

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INTRODUCTION

That soils strongly retain the orthophosphates is well recognized. These phosphates applied in dry condition move only very short distances with percolating waters. Applied in solution as in laboratory studies or under field conditions in the irrigation water, the orthophosphate ions are removed from solution and retained by the solid phase of the soil. Little is known, however, of the reactions of many other phosphorus compounds with the soil.

In connection with other studies in progress, it became desirable to know approximately how much certain organic phosphorus compounds were retained by soils and to ascertain the effect of each on plant growth. Orthophosphates as well as other inorganic phosphorus compounds were included for comparison.

METHODS

The usual method of attack in this type of investigation is to study by chemical analysis each compound in turn, its reaction with the soil, and then to determine its physiological reaction on plant growth as a separate test, later correlating the findings from the two types of tests. A more direct method was used, however, which placed its main reliance upon the physiological reaction of the plant. This method, described by Conrad and Adams (3),² is illustrated in figure 1. The plants by their enhanced growth showed the position of the phosphorus retained from a percolating solution by a soil deficient in phosphorus, if the compound in question was beneficial. If the compound was toxic, reduced growth was one criterion of retention used. The cations, calcium and sodium, it is assumed in these studies contributed little or nothing to the beneficial or toxic properties of the compounds tested.

Two lots of soils deficient in phosphorus were used in these experiments: Yolo subsoil (a loamy fine sand), C-11, collected at a depth of 4 to 8 feet from Yolo fine sandy loam on the south bank of Putah Creek, University Farm, near Davis, Calif. (4); and Aiken loam, C-22, a surface sample from near Paradise, Calif. (12).

Each 4-inch pot, previously coated with asphaltum paint, was provided with a square of waxed paper to cover the hole. Dry soil (400 gm. per pot) was then added to each. The three pots of each column were stacked with the bottom one nesting in the drainage can (No. 2½ cannery tin also painted on the inside to prevent rusting) and with the consecutive pots held apart by a cannery tin top punched

¹ Received for publication Apr. 17, 1939. Investigations of the Division of Agronomy, California Agricultural Experiment Station, Davis, Calif. The analyses reported herein were made by R. E. Malde, Division of Agronomy.

² Italic numbers in parentheses refer to Literature Cited, p. 517.

with a large hole (fig. 1). The reservoirs shown were not used in securing the data for tables 1 and 2; but they were employed for later studies. In the early work the solutions in question were poured into the top pots in successive amounts as percolation progressed.

In the comparisons made below it is assumed that practically an equal amount of liquid was retained by each pot in a given column. To test the validity of this assumption, pots were weighed both before and after percolation, and the leachings measured before being discarded or stored for analysis. Table 1 summarizes these data and gives estimates of their statistical significance. The amount of water held by a soil may, as noted elsewhere (11, p. 16), continue to increase

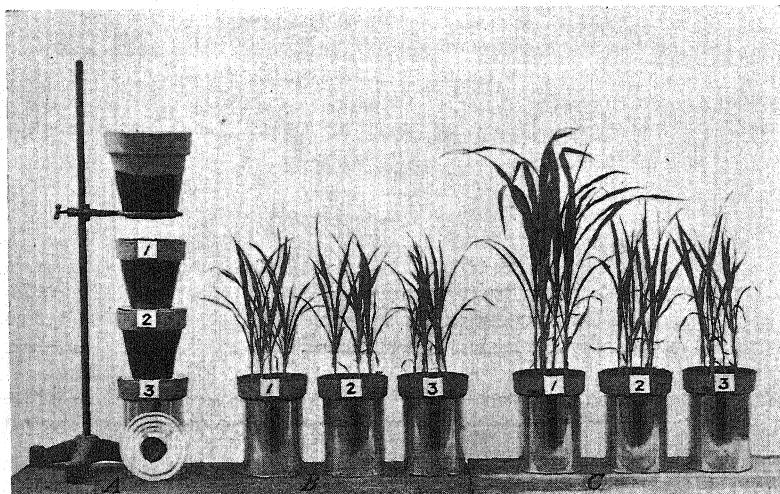


FIGURE 1.—Retention of the phosphorus from percolating solutions. At *A* the solution being tested slowly dripped from the reservoir above, down upon the column of 4-inch pots (1, 2, and 3), each containing 400 gm. of dry soil (Aiken loam) deficient in phosphorus. After percolation the pots in the column were taken down and subsequently cropped to milo. Equal amounts of nitrogen were added to each pot in the test during the growth period. The three pots at *B* were percolated with distilled water, those at *C* with a solution of Na_2HPO_4 .

for several hours as wetting of the aggregates with excess water continues. This principle explains why the water held by the middle pots was a few percent higher than that held by the bottom pots. Undoubtedly the top pots held a little more than the middle ones originally, but because of evaporation from their unprotected surfaces, the amount is unknown. With the Aiken soil in the heated greenhouse, greater evaporation would be expected from the top pots than with the Yolo soil, columns of which were percolated in the unheated head house. The water held by the top pots in these experiments was evidently less than 10 percent more than that held by the bottom ones.

TABLE 1.—Amount of percolating solution retained by the soil in the pots of the columns

Item	Yolo sub-soil, C-11	Aiken loam C-22
Columns.....number.....	18	39
Solution added per column.....milliliter.....	465	675
Solution retained:		
Pct No. 1, top.....grams.....	145	198
Pot No. 2, middle.....do.....	142	209
Pot No. 3, bottom.....do.....	137	199
Leachings, bottom pots.....do.....	20	32
Accounted for:		
Milliliters.....	444	638
Percent.....	95.5	94.5

1 Statistically different from the value below it. $P=0.01$ (5, p. 112).

2 Statistically different from the value above or below it. P much less than 0.01 (5, p. 112).

The phosphorus compounds used and the grades were as follows:

Calcium orthophosphate primary, $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$; c. p.
 Sodium orthophosphate primary, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$; c. p.
 Sodium orthophosphate secondary, $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; c. p.
 Sodium pyrophosphate, $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$; c. p.
 Metaphosphoric acid, HPO_3 —glacial sticks
 Sodium hypophosphite, $\text{NaH}_2\text{PO}_2 \cdot \text{H}_2\text{O}$; c. p.
 Calcium glycerophosphate; N. F.
 Sodium phosphite, $\text{Na}_2\text{HPO}_3 \cdot 5\text{H}_2\text{O}$
 Sodium hypophosphite; N. F. V.
 Calcium hypophosphite; N. F.
 Sodium glycerophosphate
 Sodium β -glycerophosphate
 Triethyl phosphate, $(\text{C}_2\text{H}_5)_3\text{PO}_4$

Sodium nucleate (stock solution) was made by dissolving 8.01 gm. of yeast nucleic acid in 62.5 ml. of M/2 NaOH and diluting to a liter.

EXPERIMENTS

The results of the first tests are reported in table 2. All phosphorus-containing solutions reported therein contained 2 milligram-atoms of phosphorus for each column of three pots. Since milo (*Sorghum vulgare Pers.*) seed had been planted in the dry soil, germination started with percolation. During the growth period 5 milligram-atoms of nitrogen as urea were added to each pot of the Yolo subsoil, and a total of 9 milligram-atoms as $(\text{Ca}(\text{NO}_3)_2)$ and urea to each pot of the Aiken loam. As soon as practicable the plants were thinned to six per pot. For the Yolo soil the leachings from each column were analyzed for total phosphorus. Only traces were found in any, except from the $\text{Ca}(\text{H}_2\text{PO}_4)_2$ columns. Here a solution containing 4.3 milligram-atoms of phosphorus per liter was added at the top, and the leachings at the bottom contained 3.14 milligram-atoms per liter. After 48 days for the Yolo soil and 38 for the Aiken, the green crops were harvested, weighed, dried at about 80°C . for 48 hours, and reweighed. The averages of triplicate cultures are reported in table 2.

TABLE 2.—Retention of phosphorus from various percolating solutions by phosphorus-deficient soils in columns of 4-inch pots as shown by the subsequent average yield of triplicate cultures of milo

YOLO SUBSOIL (A LOAMY FINE SAND), C-11

Pot No.	Yield per pot (grams) from percolating solution indicated							
	Control, H ₂ O (distilled)		Ca(H ₂ PO ₄) ₂ , N. F.		Calcium glycerophosphate		Ca(H ₂ PO ₄) ₂ , c. p.	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
1, Top.....	8.94	1.46	4.44	¹ 0.63	14.53	² 2.48	³ 19.11	3.28
2, Middle.....	8.61	1.50	4.98	² .63	15.26	² 2.60	9.88	1.66
3, Bottom.....	8.63	1.57	5.79	² .81	13.04	² 2.34	8.81	1.51

AIKEN LOAM, C-22

	Control, H ₂ O (distilled)		Ca(H ₂ PO ₄) ₂ , N. F.		Calcium glycerophosphate		Phytin	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
1, Top.....	4.08	0.84	3.43	0.69	14.30	³ 2.70	3.78	0.73
2, Middle.....	3.87	.81	4.01	.79	3.05	.64	3.60	.72
3, Bottom.....	4.27	.89	3.36	.68	4.23	.92	3.90	.78
	NaH ₂ PO ₄ , c. p.		NaHPO ₄ , c. p.		Na ₄ P ₂ O ₇ , c. p.		Na ₂ HPO ₄	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
1, Top.....	15.71	² 2.87	18.07	³ 3.61	10.31	⁴ 1.87	3.54	0.63
2, Middle.....	4.50	.91	4.08	.77	3.77	.74	4.30	.72
3, Bottom.....	4.57	.96	3.91	.76	3.90	.82	4.63	.85
	NaH ₂ PO ₂ , N. F.		Sodium glycerophosphate		Sodium β glycerophosphate		Sodium nucleate	
	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
1, Top.....	3.09	³ 0.57	10.06	⁴ 1.79	10.12	⁴ 1.77	8.50	⁴ 1.47
2, Middle.....	4.28	.80	4.06	.76	3.87	.71	4.46	.87
3, Bottom.....	4.09	.76	4.45	.84	3.85	.77	3.46	.76

¹ Statistically different from the control; *P* lies between 0.05 and 0.01 (5, *p.* 114).² Statistically different from the control; *P* = 0.01 or less (5, *p.* 114).³ Statistically different from the value below it; *P* = 0.01 or less (5, *p.* 112).⁴ Statistically different from the value below it; *P* lies between 0.05 and 0.01 (5, *p.* 112).

The Yolo soil retained the hypophosphite ion to only a slight degree, as shown by the toxic condition that extended rather uniformly throughout the soil column, each pot yielding significantly less than the control. The analyses of the leachings as reported in the text above are in agreement with this view. The phosphorus of the glycerophosphates penetrated significantly through the columns of the Yolo soil and into the bottom pots, but not sufficiently to evidence any increase of phosphorus in the leachings. The phosphorus of calcium orthophosphate was retained in the top pots.

From the Aiken loam columns as reported in table 2, the leachings were measured but not analyzed. Phytin, Ca(H₂PO₄)₂, and Na₂HPO₄ gave no significant differences from the controls. On the other hand, NaH₂PO₂ gave a significantly lower yield in the top pots than in the pots next below. The phosphorus of NaH₂PO₄, Na₂HPO₄, Na₄P₂O₇, and the sodium glycerophosphates as well as that of sodium nucleate was retained in the top pots by the Aiken soil, since significantly higher yields were secured there.

Some of the results given in table 2 were not decisive. If a chemical containing phosphorus gives an increase on a soil deficient in that element, it may be inferred that the phosphorus therein is responsible for the increase even though the chemical is not highly purified. When a phosphorus compound not of a highly refined grade gives a decrease on such a soil, we cannot so readily infer that the phosphorus compound is responsible, since the toxicity may be caused by some impurity of a highly toxic nature. For the work reported in table 2, hypophosphite of c. p. grade was not available; but some was secured for the work reported in later tables.

Other chemicals giving somewhat inconclusive results were used in higher concentrations than before, and the amounts of the percolating solutions increased to give ample leachings for analytical work. For growth reported in table 3, 10.4 milligram-atoms of nitrogen were added to each pot as mixed calcium, magnesium, and sodium nitrates.

TABLE 3.—Retention of the phosphorus from various percolating solutions by phosphorus-deficient soils as shown by the subsequent yield of milo and the amount of phosphorus absorbed by the plants

[Each value in the table is the average of triplicate cultures and analyses]

Pot No.	Water							
	Yolo subsoil, C-11				Aiken loam, C-22			
	Yield weights		Phos- phorus, dry	Phos- phorus in crop	Yield weights		Phos- phorus, dry	Phos- phorus in crop
	Wet	Dry			Wet	Dry		
	Grams	Grams	Percent	Milli- grams	Grams	Grams	Percent	Milli- grams
1, Top.....	4.21	0.89	0.06	0.54	3.38	0.68	0.06	0.41
2, Middle.....	3.95	.80	.057	.46	3.63	.73	.065	.48
3, Bottom.....	3.86	.80	.06	.48	3.55	.75	.06	.45
Leachings (milligram-atoms of P per liter).....		.006	-----			.006	-----	
Phytin, 4 ¹ milligram-atoms								
1, Top.....	² 8.94	² 1.44	0.17	² 2.45	4.96	0.94	0.08	0.75
2, Middle.....	4.00	.84	.09	.76	3.22	.65	.07	.45
3, Bottom.....	4.05	.84	.07	.58	3.31	.66	.065	.43
Leachings (milligram-atoms of P per liter).....		.03				.01		
HPO ₃ , 8 ¹				NaH ₂ PO ₂ , c. p., 4 ¹				
1, Top.....	4.47	0.75	0.21	1.58	3.66	0.62	0.11	0.68
2, Middle.....	9.68	1.41	.197	¹ 1.78	3.72	.66	.10	² .66
3, Bottom.....	4.18	.88	.08	.70	3.76	.73	.055	.40
Leachings (milligram-atoms of P per liter).....		² .03				² .006		
Na ₄ P ₂ O ₇ , c. p., 4 ¹				Ca(H ₂ PO ₄) ₂ c. p., 4 ¹				
1, Top.....	¹ 7.72	¹ 1.28	0.33	¹ 4.23	¹ 24.73	¹ 3.23	0.30	¹ 9.69
2, Middle.....	3.80	.79	.07	.55	3.79	.78	.07	.55
3, Bottom.....	4.11	.83	.07	.58	4.05	.81	.07	.57
Leachings (milligram-atoms of P per liter).....		² .02	-----			² .006	-----	

¹ Milligram-atoms of phosphorus.

² Statistically greater than the value below it. $P=0.01$ or less (5, p. 112).

³ Statistically greater than the value below it. P lies between 0.05 and 0.01 (5, p. 112).

Just before harvest, it was evident that the growth of the test plants alone would not conclusively give the location of the phosphorus in all the columns. In the most inconclusive cases the plants were weighed, dried, weighed, and then analyzed for total phosphorus. The results are reported in table 3 as "Phosphorus in crop." The concentration of phosphorus in the leachings is also given. In all cases reported in table 3 except the controls, the concentration of phosphorus in the leachings was not greater than 0.5 percent of that in the percolating solution.

The Yolo soil retained the phosphorus of phytin and of $\text{Na}_4\text{P}_2\text{O}_7$ in the top pots. The phosphorus of metaphosphoric acid, HPO_3 , penetrated in statistically significant quantities to the second pot, with a suggestion of penetration to the third, as shown by the analyses of the crops grown on them. The growth from the top pot was practically equal to that with distilled water and was less than half that of the second pot. The yield alone is not a true criterion of the location of the phosphorus. There is no rational means by which all the phosphorus could pass through the top pot and become concentrated in the middle one. The most obvious explanation is that either hydrogen ions or metaphosphate ions or the resultant products of the reactions of each or both in the soil were retained in sufficient quantity in the top pot to just balance the toxic and beneficial effects.

TABLE 4.—Retention of the phosphorus from various percolating solutions by phosphorus-deficient soils as shown by the subsequent green growth of milo and the phosphorus content of the leachings collected from the bottom of the columns

YOLO SUBSOIL, C-11

Pot No.	Yield per pot (grams) from percolating solution indicated ¹						
	H_2O ²	Na_2HPO_4 ² (12.9)	NaH_2PO_4 ² (6.45)	$(\text{C}_2\text{H}_5)_3\text{PO}_4$ ³ (12.9)	H_2O ⁴	$(\text{C}_2\text{H}_5)_3\text{PO}_4$ ⁴	
						0.97	4.83
1, Top.....	4.21	⁵ 1.45	⁶ 0.58	⁶ 0.00	4.22	2.54	⁶ 0.10
2, Middle.....	3.95	⁵ 6.98	⁶ 6.61	⁶ 6.00	4.25	2.46	⁶ 3.73
3, Bottom.....	3.86	⁵ 1.25	⁶ 6.74	⁶ 6.32	3.97	4.00	⁶ 2.19
Leachings per liter.....	1.006	¹ 2.9	¹ 6.3	¹ 0.22			

AIKEN LOAM, C-22

Pot No.	H_2O ²	Na_2HPO_4 ² (8.9)	HPO_3 ³ (8.9)	$(\text{C}_2\text{H}_5)_3\text{PO}_4$ ³ (8.9)	H_2O ⁴	$(\text{C}_2\text{H}_5)_3\text{PO}_4$ ⁴	
						0.67	3.33
1, Top.....	3.38	⁵ 1.72	⁷ 11.41	⁶ 0.00	2.29	2.06	⁶ 0.88
2, Middle.....	3.63	3.65	3.72	⁶ 0.00	2.37	1.92	⁵ 1.10
3, Bottom.....	3.55	3.63	2.41	⁶ 0.00	2.17	1.92	⁵ 1.16
Leachings per liter.....	1.006	1.006		¹ 0.29			

¹ As milligram-atoms of phosphorus per liter.

² Mixed calcium, magnesium, and sodium nitrates added; 10.4 milligram-atoms of nitrogen per pot.

³ Mixed nitrates added; 10.0 milligram-atoms of nitrogen per pot. 3 pots each for Yolo subsoil averaged 2.65 gm. and for Aiken loam, 2.41 gm. These received no phosphorus and were not subject to percolation, but otherwise were treated like those reported above.

⁴ Mixed nitrates added; 7.4 milligram-atoms of nitrogen per pot. In this series of tests 1 column of each soil was percolated with H_2O ; 2 with the lower and 3 with the higher concentrations of $(\text{C}_2\text{H}_5)_3\text{PO}_4$ (triethyl phosphate). All other values in this table are the average of triplicate cultures and analyses.

⁵ Statistically different from the control; P lies between 0.05 and 0.01 (δ , p. 114).

⁶ Statistically different from the control; $P=0.01$ or less (δ , p. 114).

⁷ Statistically different from the value below it; $P=0.01$ or less (δ , p. 112).

⁸ Statistically different from the value below it; P lies between 0.05 and 0.01 (δ , p. 112).

Aiken loam did most obviously retain the phosphorus of phytin, but in a form only slightly, if at all, accessible to the test plants. The yields of the top pots, however, just failed to be significantly higher than those of the middle ones (P =about 0.06). The c. p. grade of NaH_2PO_2 did not significantly show its toxicity for milo on Aiken loam. The analyses of the plants, however, gave significant evidence that the phosphorus of this compound had penetrated into the second pot of the column.

In table 4 the data for Na_2HPO_3 and NaH_2PO_2 with the Yolo soil and for the Na_2HPO_3 with the Aiken soil were obtained at the same time as those for table 3. Subsequent trials, as reported in table 4, were with various concentrations of triethyl phosphate on both soils and with HPO_3 on Aiken loam. Some of the responses in growth to

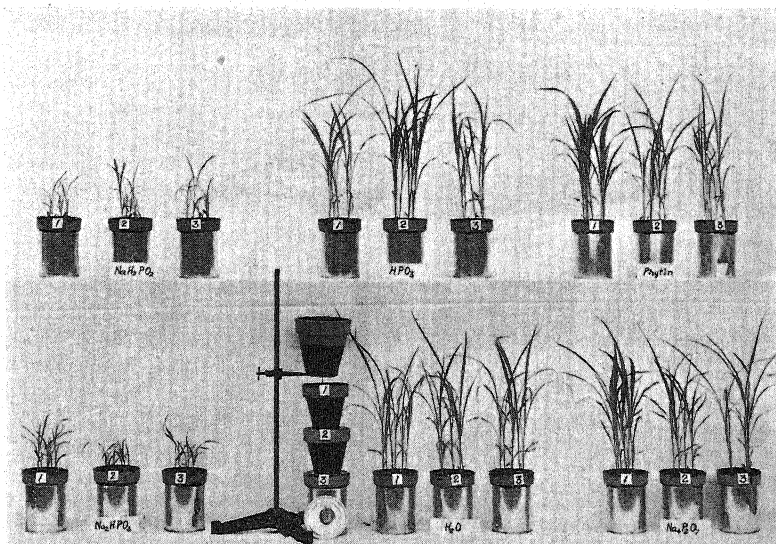


FIGURE 2.—Retention of phosphorus from various solutions by Yolo subsoil, C-11. Enhanced growth in some cases and reduced growth in others were important criteria of retention (cf. tables 3 and 4).

various phosphorus-containing solutions reported in tables 3 and 4 are shown in figure 2 for Yolo subsoil and in figure 3 for Aiken loam.

The evidence shows that the phosphorus of Na_2HPO_3 was retained to some extent by the Yolo soil, since the concentration of phosphorus in the leachings was but 25 percent of that of the original solution. The phosphorus of NaH_2PO_2 was retained hardly at all. The toxicity of Na_2HPO_3 and NaH_2PO_2 extended significantly down through the soil columns.

With Aiken loam the evidence indicates that most of the Na_2HPO_3 was retained in the top pot. The phosphorus of HPO_3 did not significantly penetrate beyond the top pot, though there was a suggestion (P =0.2) that some had reached the middle pot. Triethyl phosphate was retained to a certain extent by both soils, but somewhat more strongly by the Yolo soil. It was, furthermore, toxic to plant growth

—more so on the Aiken than on the Yolo soil, especially with the highest concentrations used.

DISCUSSION

Table 5 gives an estimate of the final percentage distribution of the phosphorus of the original percolating solution, assuming that the solvent was distributed in four equal parts among the three pots and the leachings. Increases and decreases in yield, the analyses of the

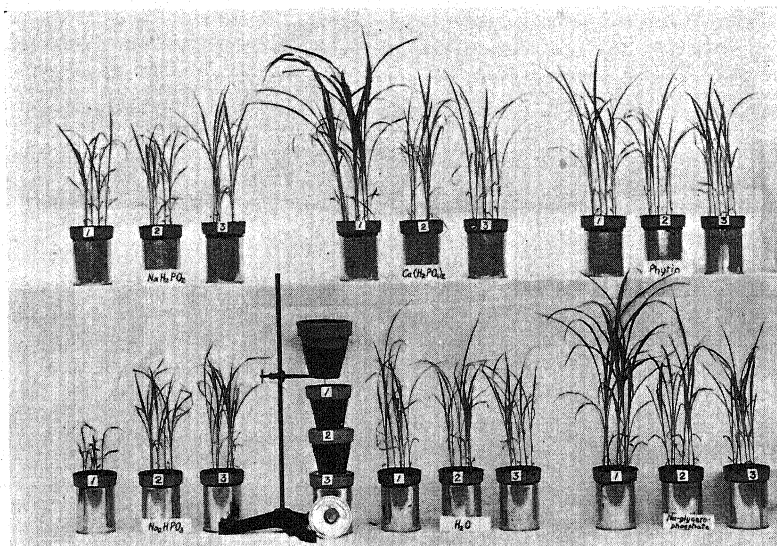


FIGURE 3.—Retention of phosphorus from various percolating solutions by Aiken loam, C-22. Retention is judged in part by the enhanced or reduced growth of milo in the top pots (cf. tables 3 and 4). The lot of Aiken soil used for sodium glycerophosphate and for the corresponding controls received a slightly different nutritional treatment. The top, middle, and bottom pots of the controls averaged for green weights 4.40, 4.77, and 4.81 gm. respectively, while those for the sodium glycerophosphate averaged 18.34, 4.88, and 4.54 gm. respectively. The yields from the top pots of the sodium glycerophosphate columns differed from the others very significantly. None of the other differences, however, were significant.

leachings, and the principle that no pot could retain a higher percentage of the original phosphorus than a pot above it in the same column were used as bases of estimation. Undoubtedly, reestimating of the data would result in slightly different figures in a few cases, but the relationships would not be materially changed. The possibility of more accurately estimating the location of the phosphorus compounds, by increasing the number of pots in the column was not explored in this study.

TABLE 5.—Summary of retention data as estimated final percentage distribution of the phosphorus of the original solutions

Chemical source	Percentage retention of phosphorus by—							
	Yolo subsoil, C-11				Aiken loam, C-22			
	Pot No. 1	Pot No. 2	Pot No. 3	In leachings	Pot No. 1	Pot No. 2	Pot No. 3	In leachings
NaH_2PO_4	28	26	24	22	70	30	0	0
Na_2HPO_4	40	30	23	7	90	10	0	0
HPO_3	75	25	0	0	90	10	0	0
$\text{Na}_4\text{P}_2\text{O}_7$	100	0	0	0	100	0	0	0
Na_2HPO_4					100	0	0	0
NaH_2PO_4					100	0	0	0
$\text{Ga}(\text{H}_2\text{PO}_4)_2$	100	0	0	0	100	0	0	0
Glycerophosphates.....	38	36	26	0	100	0	0	0
Phytin.....	100	0	0	0	100		0	0
Triethyl phosphate.....	45	35	20	0.1	43	35	21	1
Sodium nucleate.....					90	10	0	0

In seeking to discover the mechanisms by which the different phosphorus-containing units are retained one should consider the following factors:

(1) *Precipitation in the soil.*—If the anions of the percolating solution form a very difficultly soluble salt with any cations that might be liberated from the soil colloids and made soluble by exchange with the cations of the original solution, precipitation would be expected. Although many salts are theoretically possible, from a practical standpoint few besides the calcium and magnesium salts require attention. Since no hypophosphites are insoluble, the fact that the phosphorus was nearly equally divided in the Yolo subsoil would be expected. Since the hypophosphites were retained rather strongly by the Aiken soil, some other mechanism must be sought. Calcium and magnesium phosphites are only slightly soluble. From a qualitative standpoint, then, some of the retention of phosphites might be by precipitation. Since the Yolo soils are neutral and the Aiken soils are distinctly acid (about pH 5.8), it is difficult to explain the greater retention of phosphites by the Aiken soil on the basis of precipitation alone. Calcium metaphosphate is difficultly soluble, whereas the magnesium salt is soluble. Retention might be expected from the formation of the calcium salt by cationic exchange. The same might be said for the pyrophosphates and orthophosphates.

Since none of the glycerophosphates likely to be formed in the soil are insoluble, the strong retention of the phosphorus of glycerophosphates by the Aiken soil must be explained on other grounds.

Phytin, the calcium (sometimes some magnesium is present) salt of phytic acid, is soluble. Nucleic acid itself is listed as insoluble in water (?), and magnesium nucleate is also given as insoluble. Retention of the phosphorus of sodium nucleate, as shown by plant growth, might then be due to the formation of magnesium nucleate as the sodium exchanges with magnesium, a replaceable cation.

Triethyl phosphate, an ester of ethyl alcohol and phosphoric acid, miscible with water in all proportions, would not have a chance, as far as we know, to form an insoluble salt if it remained unchanged.

(2) *Adsorption or anionic exchange.*—Undoubtedly, other major factors in the retention of the orthophosphate ions by soils might be the phenomena of anionic exchange between the percolating solution and the solid phase of the soil and adsorption of the whole molecule by the solids in the soil as suggested by work reviewed by Russell (9) and more clearly indicated by later evidence (1, 2, 8). The data herein show some retention of the triethyl phosphate, an ester without ionic properties. Unless marked hydrolysis of this compound has taken place during percolation, this phenomenon suggests adsorption of this ester as whole molecules by the solid phase of the soil. To what extent the same types of phenomena were effective in causing retention of pyrophosphates, metaphosphates, phosphites, and hypophosphites is as yet unknown. If the hypophosphites remain as such, adsorption (or anionic exchange) seems to be the only process by which these phosphorus-bearing units could be retained. If such widely different compounds as hypophosphites (with Aiken loam) and orthophosphates with soils generally are adsorbed, one may suppose that compounds with intermediate properties could also be so retained.

(3) *Transformations to retainable chemical forms by hydrolysis, oxidation, or other chemical change.*—The possibility of the change from one form to another during the actual process of percolation cannot be neglected. If a mechanism for such a transformation (during the time when the solution itself is moving through the soil) exists in one soil and not in another, differences in behavior might necessarily result. More evidence is necessary before this factor can be given major consideration. The hypothesis that either the hypophosphites or the phosphites are transformed to orthophosphates and retained as orthophosphates, seems untenable because of the toxicity of the reduced forms of phosphorus as contrasted with the enhanced growth resulting from the phosphates, unless, of course, at the same time other toxic materials are formed thereby.

Some readers may question whether the differences in retention of the various phosphorus compounds found in these two soils may not be attributable to a difference in soil class since one is a loamy fine sand and the other a loam, and the loam, which has a higher amount of colloid, is for the most part more retentive. Undoubtedly, this is a factor of importance. Two pieces of evidence point, however, to other factors as well. The much lighter soil, the Yolo, retained triethyl phosphate more readily than did the Aiken soil, and the crop on the Yolo was less injured. Again, 75 percent of the glycerophosphate was leached by Spencer and Stewart (10) from a 7-inch depth of Las Vegas loam with a 24-inch depth of water. This is the same type of behavior observed with the Yolo soil; but the Aiken soil, by retaining the glycerophosphate in the top pot, seems to exemplify another type of behavior.³ This difference in behavior may later be associated with a difference in the type of soil colloids or with other factors as yet not evaluated.

³ While this manuscript was being prepared a paper by Hilbert *et al* (6) came to the writer's attention. Using the methods of analytical chemistry on the leachings, they found differences similar to those here reported in the behavior of glycerophosphates on various soils. The writer's Yolo subsoil, like their Norfolk sandy loam and Las Vegas loam, retained the phosphorus of the glycerophosphates only weakly, whereas the Aiken loam, like their Cecil clay loam, retained it very strongly.

The data presented herein answer in a qualitative or semiquantitative manner certain questions concerning the interaction of these phosphorus compounds with the soils studied. The evidence, however, serves to raise many other questions, especially regarding the various mechanisms that may be responsible for the results secured. Without a doubt, the reactions of each chemical with the soil might very well be made the subject of a special detailed study.

SUMMARY

Each phosphorus compound in solution was allowed to percolate through a stack or column of three pots containing dry soil deficient in phosphorus. The volume of solution was sufficient to wet all the soil, but without great excess. If the compound was beneficial, the enhanced growth of the subsequent crop of milo was considered as evidence of retention; if toxic the reduced growth was one criterion of retention. Analyses of the crop and of the leachings were other criteria sometimes used.

Of the inorganic compounds tested, Yolo subsoil showed little retention of hypophosphites, but somewhat more for phosphites. The phosphorus of metaphosphoric acid reached the second pot, but that of the pyro- and orthophosphates was held in the top pots. The Aiken soil for the most part held each inorganic compound more strongly than did the Yolo.

Of the organic compounds tested, the Yolo soil retained the phosphorus of phytin in the top pot, but held back the glycerophosphates only slightly. The Aiken soil retained the phosphorus of nearly all these compounds as well as that of sodium nucleate, in the respective top pots. Triethyl phosphate, held back somewhat by both soils, was more strongly retained by the Yolo and was more toxic on the Aiken.

The phosphites and hypophosphites were toxic to milo. The meta-, pyro-, and orthophosphates (if not too concentrated) and the glycerophosphates were beneficial to both soils, with phytin beneficial on the Yolo soil and sodium nucleate on the Aiken soil.

The possible mechanisms by which the various compounds were retained are discussed, including chemical precipitation in the soil, either adsorption or anionic exchange or both, and chemical transformations (during the actual process of percolation) to some other forms retained by the soil.

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PRODUCTION OF GROWTH SUBSTANCE ON PEPTONE BROTH BY CROWN GALL BACTERIA AND RELATED NONGALL-FORMING ORGANISMS¹

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INTRODUCTION

The physiology of crown gall development has been the subject of numerous studies in the general field of pathological cell growth. The recent work with phytohormones has stimulated investigation about their relation to crown gall development. The ability of the crown gall organism, *Phytoplasma tumefaciens* (Smith and Town.) Bergey et al. to produce growth substance when cultured on certain media (1, 3, 8)³ has been demonstrated, and more than the normal amount of growth substance was found in gall tissue (9). Thus, the possibility that the growth substance, if produced abundantly in the tissues, may be the cause of the pathogenicity, as suggested, for example, by Nemec (12), Brown and Gardner (3), and Link, Wilcox, and Link (8), and as claimed by Berthelot and Amoureux (1), appeared worth further study.

The production of beta-indole-acetic acid in bacterial cultures is no recent discovery. Long before it was identified with a plant-growth substance (7) it was isolated from bacterial cultures by a number of workers (5, 15). Following the development of the *Avena* technique for quantitative estimation of this product (16) several workers undertook the study of growth-substance production by bacteria (2, 14). These investigations indicate that the ability to produce growth substance in culture is widespread among such organisms. Thus it seemed that a study of the production of beta-indole-acetic acid by virulent crown gall bacteria in comparison with that by attenuated crown gall and nonpathogenic bacteria might shed light on the relation of this substance to pathogenicity. This paper, of which a preliminary report was given earlier (10), presents the results of experiments on the relative abilities of virulent crown gall bacteria, attenuated crown gall, and *Bacillus radiobacter* Beij. and Van Deld. to produce growth substance in peptone broth.

METHODS

The organisms employed were the progeny of single-cell isolations. The virulent crown gall (A6) and attenuated crown gall (A6-6) are sister cultures obtained from a single-cell virulent culture (4). The response of tomato (*Lycopersicon esculentum* Mill.) to inoculation with the attenuated culture is very slight. While the responses of several other plants, e. g., *Bryophyllum pinnatum* (L.) Kurz, *Kalanchoe*

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² The writers are indebted to Eugene Herrling for preparing the illustration.

³ Italic numbers in parentheses refer to Literature Cited, p. 524.

daigremontiana Hamet and Perrier, and *Sedum spectabile* Bor., are greater than that of tomato to the attenuated form, they are always much less than the response of the same hosts to the virulent culture (11). *Bacillus radiobacter* (R3-1) was included because it resembles *Phytophthora tumefaciens* physiologically, but produces no overgrowths whatsoever when inoculated into the plants mentioned above.

The medium employed in the cultures of the organisms was made up as follows: Parke, Davis' Bacteriologic Peptone, 10 gm.; Armour's Beef Extract, 3 gm.; mannitol, 5 gm.; and distilled water, 1,000 cc. In one case (trial 3), 40 mg. of 1-tryptophane per liter was added. The hydrogen-ion concentration was adjusted to pH 7.0 with N/20 NaOH and the medium was distributed in 250-cc. portions into 1-liter Erlenmeyer flasks which were then plugged and sterilized for 1 hour at 15 pounds pressure.

The flasks were seeded with a 3-mm. loopful from a 48-hour tube culture on the medium described. The cultures were carried in duplicate in each trial and two unseeded flasks served as controls. The cultures were incubated at laboratory temperature in all but the final trial, in which incubation was at 23°-24° C.

Determinations of growth substance were made in red light at 23°-25° C. and over 90-percent relative humidity in accord with Went's standard technique (17). Beginning shortly after seeding and extending through a period of 2 or three weeks, the samples were drawn at intervals aseptically from each flask. Without concentration, each sample was mixed with an equal volume of melted 3-percent agar and the mixture was poured into a mold 1 by 8 by 11 mm. from which 12 equal blocks were cut. These were placed unilaterally on 12 decapitated *Avena* coleoptiles which were prepared as follows: The oats, variety State's Pride, Pedigree 7-7, were placed in water at 9 o'clock each morning. After 1 to 2 hours the water was poured off and the oats were hulled and placed, embryo up, upon an aluminum wire screen which covered a small dish filled with water so that the screen touched the surface of the water at all points. The seeds were allowed to germinate in red light at 23°-25° C. until the second morning, when the coleoptiles were about 5 mm. long. They were then placed in glass holders, 12 to a rack, and, with their roots in water, were allowed to develop 1 day longer. At this time those having coleoptiles between 25 and 30 mm. in length were selected and decapitated about 2 mm. below the tip. Three hours later they were decapitated a second time and the first leaf was pulled loose from the base and left projecting about 5 mm. from the top of the decapitated coleoptile. The agar blocks containing the growth substance were placed on one side of the decapitated coleoptile, being supported in this position by the projecting first leaf. Good contact between block and coleoptile stump was obtained by touching the bottom of the block with a small drop of water. Ninety minutes after applying the blocks a shadow print of each rack of coleoptiles was made. When growth substance was present in the blocks the coleoptiles curved away from the side on which the block was applied because of the greater growth of that side of the coleoptile. This curvature was measured on the shadow prints by means of a protractor and the average curvature of 12 coleoptiles was taken as a measure of the growth substance present in a given preparation. In the final trial a standard solution of known activity was employed

daily to determine the sensitivity of the coleoptiles on that particular day (13, 17). In this case it was possible to express the results in gamma per liter of beta-indole-acetic acid.

EXPERIMENTAL RESULTS

The 4 trials reported in table 1 were made during a period extending from January 1 to September 17, 1938, and involved the use of 5,160 coleoptiles. In every trial, growth substance was produced in considerable quantity in all three cultures, while curvatures obtained with the unseeded medium were relatively small. Differences among curvatures obtained with samples from the different cultures were too small to be considered significant. Since the rate of production and the time required to reach a maximum concentration were different in the several trials, the results of each series are discussed separately. The summary of data obtained in these trials are given in table 1. In addition to the 4 series mentioned above, 3 other trials were made involving the use of 2,112 coleoptiles. While these gave results paralleling those reported, they are omitted for the sake of brevity and because of slight modifications in procedure.

TABLE 1.—Summary of 4 trials in which the production of growth substance on peptone broth by *Bacillus radiobacter*, attenuated crown gall bacteria, and virulent crown gall bacteria was determined

Trial No.	Culture	Negative curvature obtained after indicated number of days ¹															
		0	1	2	3	4	5	6	7	9	10	11	14	17	19	21	24
1	Unseeded medium	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	<i>Bacillus radiobacter</i>				9				1			1	1	1			4
	Attenuated crown gall				12				6		6		14	18			27
	Pathogenic crown gall				9				9		7		15	18			27
2	Unseeded medium	2			4					5		3			1		
	<i>Bacillus radiobacter</i>	4			6			25		26		27			28		
	Attenuated crown gall	5			7			26		26		26			29		
	Pathogenic crown gall	4			8			23		24		32			25		
3	Unseeded medium	4	0	2	2	1	1	2	0	1	1	2	4				
	<i>Bacillus radiobacter</i>		4	6	5	8	18	16	16	9	9	12	8				
	Attenuated crown gall		6	7	7	9	16	16	10	11	8	12	13				
	Pathogenic crown gall		4	2	5	10	14	16	11	10	12	12	14				
4	Unseeded medium	1			3	3	3		3	2		3	1	1			4
	<i>Bacillus radiobacter</i>		2		4	5	11		8	6		8	9	9			12
	Attenuated crown gall		2		2	5	8		9	8		5	8	8			10
	Pathogenic crown gall		1		3	2	6		6	5		6	8	12			11

¹ Each figure represents the average of curvatures produced in 12 *Avena* coleoptiles.

Trial 1 was begun January 31 and was completed February 24, 1938. Following a slight decrease in growth-substance curvatures from the third to the seventh day there was a gradual increase throughout the duration of the experiment, a maximum of about -27° being reached on the twenty-fourth day after seeding, when the experiment was terminated. Curvatures produced by unseeded medium dropped to near -1° on the seventh day and remained there throughout the rest of the experiment except for the final determination, when a curvature of approximately -4° was obtained.

Trial 2 was begun March 7 and concluded March 26. Growth-substance curvatures in all three cultures increased slowly during the first 3 days to about -7° , then rapidly until the sixth day to about -25° , and then very slowly until a maximum of about -30° was

reached on the nineteenth day when the experiment was terminated. The curvatures produced by the unseeded medium were close to -5° for the first 9 days, then gradually decreased to nearly 0° on the nineteenth day.

Trial 3 was begun August 1 and concluded August 15. To the basic medium, 40 mg. of 1-tryptophane per liter was added. After a lag period of 3 days the curvatures produced by all three cultures increased rapidly to a maximum of about -18° and -16° on the fifth and sixth days respectively. This was followed by a decrease to about -10° in the next 3 days, and then by an increase to about -12° by the eleventh day. On the fourteenth day the crown gall cultures gave about -13° while the *radiobacter* culture gave a curvature of about -8° . This is not considered a significant difference, especially since it may be attributed to the fact that the determination was made just after the activity of the *Bacillus radiobacter* culture had dropped, but shortly before a similar decrease in activity occurred in the crown gall cultures. The unseeded medium gave curvatures of less than -4.5° throughout the experiment.

Trial 4 was begun August 27 and terminated September 17. The curvatures produced by the cultures showed a lag period of from 3 to 4 days, which was followed by a period of rapid increase reaching a maximum of approximately -6° to -11° on the fifth day. From the seventh to the ninth there was a decrease in the curvatures to between -5° and -6° . This was followed by a more gradual increase in curvatures to about -11° on the twenty-first day when the experiment was discontinued. The curvatures produced by the unseeded medium at no time exceeded -4.5° . A standard solution was tested on each lot of coleoptiles employed during this trial so that it is possible to express the activities of the samples in terms of concentrations of beta-indole-acetic acid giving the same curvatures (fig. 1).

While of minor importance, the bimodal character of the curves obtained in trials 3 and 4 is of interest, and can be explained in several ways. For example, while the cultures are young they probably draw mainly upon the sugar present as a source of carbon, which has a sparing action on the peptone. However, as the cultures age they probably use the nitrogenous compounds as sources both of carbon and of nitrogen. These changes in nutrition could bring about a change in growth-substance concentration (1) by altering the reaction of the medium and thus the stability of the growth substance, or (2) by altering the kind and quantity of compounds present from which the growth substance could be formed.

The possibility was considered that the differences in pathogenicity of the organisms employed may be attributed to qualitative rather than quantitative differences in the growth substances produced. So, the stabilities of these growth substances toward acid and base were tested. The growth substances from all three cultures proved to be unstable in acid and stable in basic solution, indicating that they belong to the indole acids, probably beta-indole-acetic acid (6).

DISCUSSION AND CONCLUSIONS

No significant differences were found among the three organisms employed with respect to their ability to produce growth substance when grown on peptone broth, notwithstanding the fact that they

differ widely in their ability to induce overgrowths when inoculated into plants susceptible to crown gall. The possibility still remains that the formation of growth substance by the crown-gall organism from

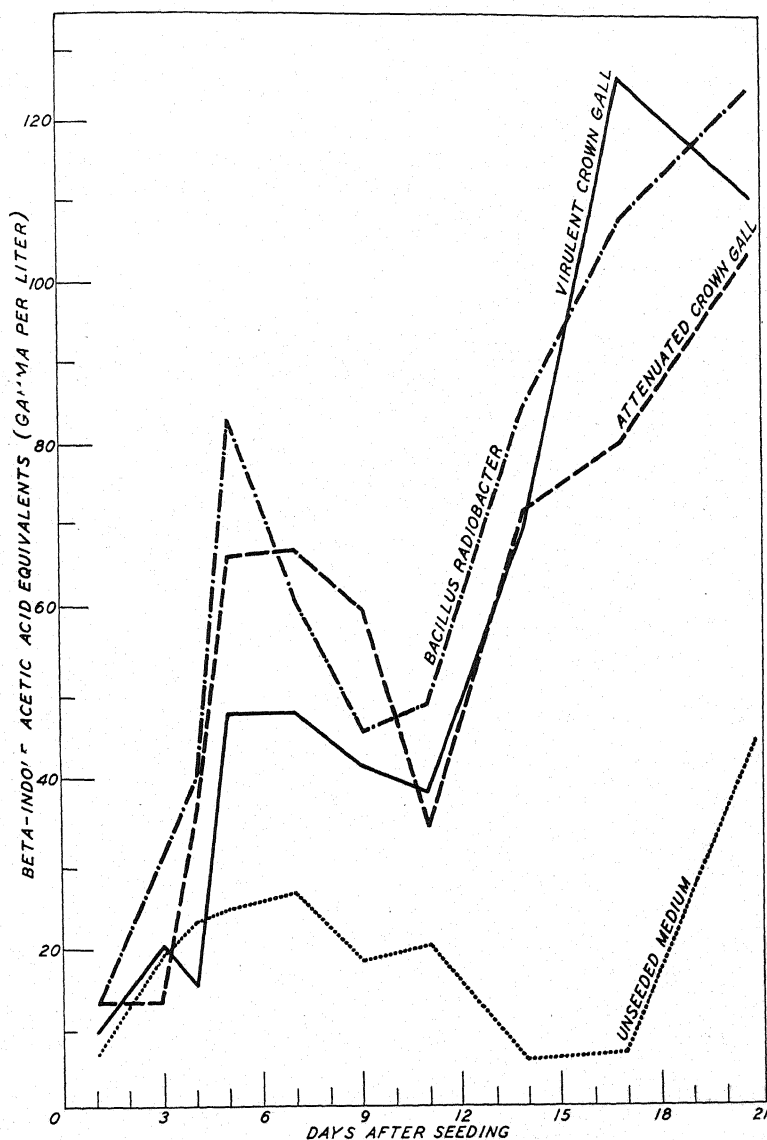


FIGURE 1.—Heteroauxin concentration in unseeded peptone broth and in virulent crown gall, attenuated crown gall, and *Bacillus radiobacter* cultures.

the materials available to it within the host tissues may constitute a part of the mechanism leading to excessive growth. However, the possession of this ability does not yet explain the differences in pathogenicity among the organisms employed in these experiments. As pointed out earlier (9, 11), the difference in pathogenicity between the virulent and the attenuated crown-gall cultures seems not to be ex-

plained by a differential bacteriostatic effect of the host. Consequently, evidence seems lacking that the growth substance produced in culture has a direct major relation to the pathogenicity of crown gall-bacteria.

SUMMARY

Single-cell bacterial cultures of three organisms (virulent crown gall, attenuated crown gall, and *Bacillus radiobacter*) differing widely in ability to induce overgrowths in plants, but similar in physiology, were found to be similar in their capacity to produce growth substance in peptone broth. Thus far evidence seems lacking for the view that beta-indole-acetic acid or any other known growth substance produced in such cultures has a direct major relation to the pathogenicity of crown-gall bacteria.

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SPORULATION AND VIABILITY OF OÖCYSTS OF *EIMERIA ARLOINGI* FROM THE DOMESTIC SHEEP¹

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INTRODUCTION

Although a considerable amount of general information is available concerning the factors that influence the sporulation and viability of oöcysts of sheep coccidia, more specific knowledge is needed for a logical approach to the problem of control of coccidiosis through prevention.

Most of the available facts concerning the behavior of oöcysts of ovine coccidia are attributable to the work of Lerche.² Other investigators, namely, Douwes,³ Yakimoff and his colleagues,⁴ and Carré,⁵ have largely confirmed and expanded the conclusions of Lerche. From the work of these investigators, it is known that the oöcysts never sporulate inside the intestine of the host because of the prevailing anaerobic conditions; that oöcysts discharged from the host in fecal pellets sporulate within 2 to 3 days, provided conditions of temperature, moisture, and oxygen tension are optimum; that excellent conditions for sporulation often occur in the litter of the fold and in protected sites in pasture grass; that putrefaction and drying are destructive to oöcysts, whereas cold retards the rate of sporulation; and that infection of a new host takes place through ingestion of feed and drink contaminated with fecal material containing sporulated oöcysts.

The general nature of these conclusions may be attributed to lack of detailed experimental work in which all factors have been rigidly controlled. A precise knowledge of conditions favorable or inimical to sporulation is desirable in order to facilitate the recognition and elimination of specific locations in pastures and yards that are favorable to the sporulation and preservation of oöcysts. It is also of practical importance to know how long sporulated or unsporulated oöcysts will retain their viability under given conditions. Conclusions derived from data on sporulation and viability experiments constitute the best basis for formulating measures for the prevention and control of coccidiosis.

The present study was undertaken in order to define more accurately the conditions that favor, retard, or prevent sporulation and destroy or preserve viability of the oöcysts. Although the generalizations presented probably apply equally to all species of ovine coccidia, the data were obtained entirely from experiments on oöcysts of *Eimeria arloingi* Marotel, which occur in greater abundance and frequency

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⁵ CARRÉ, H. LA COCCIDIOSE DU MOUTON. Rec. Méd. Vét. 104: 530-539. 1928.

than those of other species from sheep. An attempt was made in the experimental procedures to duplicate conditions that actually exist in the fold and the pasture, in order to make the results directly applicable to conditions existing in the natural environment.

MATERIALS AND METHODS

Freshly discharged oöcysts of *Eimeria arloingi* were obtained from fecal pellets from several 4- to 8-week-old lambs kept near the laboratory of the Zoological Division of the Bureau of Animal Industry, at Beltsville, Md., where this investigation was conducted. Almost daily fecal examinations were made on a total of nine lambs, four during April 1937, and five late in March and early in April 1938. The lambs, with their mothers, were kept in an enclosure 50 feet long by 20 feet wide, and their milk diet was supplemented with water, grain, and hay. Tap water was supplied in a tub, the grain was fed in an open trough upon the ground, and the hay was scattered directly upon the ground. The lambs and ewes had access to the same feed and bedding straw, thereby providing excellent opportunities for the transmission of the infection to lambs from adult animals, the latter being latent carriers.

Few or no oöcysts were discharged by the lambs during the first 3 or 4 weeks after birth, but during the succeeding several weeks enormous numbers were passed. During this period of high oöcyst discharge there was usually a significantly high peak lasting several days during the fourth or fifth week after birth, followed by a lower grade, fluctuating discharge for the next few weeks. Fecal samples were obtained by isolating lambs inside individual, plank-floored pens until pellets were dropped. After release of the animals and collection of samples, each pen was carefully swept, locked, and dried until the next day. Fecal specimens containing oöcysts in sufficient numbers for sporulation experiments could be obtained at any time during the period of high oöcyst discharge.

In the fold and the pasture, discharged fecal pellets containing oöcysts may be subjected to the following conditions: (1) They may fall into running water, which washes the oöcysts relatively free from contaminating organic debris; (2) they may fall into stagnant water and form a layer of putrefying organic sediment on the bottom, in which the oöcysts are lodged; (3) they may fall upon and percolate into litter straw, hay, grass, or grain in feed boxes, where the oöcysts find favorable conditions of moisture for the preservation of viability for indefinite periods; or (4) they may fall in open places exposed directly to the drying action of sunshine and wind. The fecal pellets are exposed to temperature fluctuations, as well as to these natural environmental conditions. In laboratory tests, these possible environments for oöcysts were duplicated as nearly as possible, and each preparation was subjected to several temperatures within the range of those occurring in the natural environment.

To obtain oöcysts free from contaminating debris for sporulation tests in clean water, fresh fecal pellets were crushed in a mortar, mixed with water, and the mixture washed with more water through a 30-mesh sieve into glass containers for settling. After at least an hour of sedimentation, the supernatant fluid was poured off and 1-cc. lots of the sediment were thoroughly mixed with 14-cc. lots of 35-percent sugar solution in 15-cc. centrifuge tubes, which were then allowed to stand for another hour to permit flotation of the oöcysts.

By lifting off the center of the surface film of each tube with a wire loop, the oöcysts were removed from the tubes relatively free from fecal debris. These loopfuls containing oöcysts were placed upon the bottom of the spherical pits of 25- by 75-mm. culture slides, and clean tap water was added to fill the depressions. Oöcysts soon settled to the bottom of the pits and lay beneath about 2 mm. of clean water, with little contaminating debris to obstruct vision or cause putrefaction. The slides were labeled, placed inside moist chambers made by adding water to the floors of Petri dishes, covered, incubated at the desired temperatures, and removed at intervals to determine evidence of sporulation.

A putrefying organic environment was created by placing fecal sediment containing oöcysts in open dishes to depths of about 15 mm., and keeping it barely covered with water by periodic additions during incubation at different temperatures. At room and incubator temperatures it was also necessary to place preparations inside improvised moist chambers to prevent drying. To check for evidence of sporulation, quantities of the sediment were mixed with sugar solution and the floated oöcysts were transferred to slides with a wire loop and observed.

The ability of oöcysts to sporulate inside pellets in which the natural amount of fecal moisture is retained was tested by placing fresh pellets upon a layer of soaked filter paper inside closed Petri dishes and incubating at the desired temperatures. The wet paper insured a saturated atmosphere within the dish, thus preventing loss of fecal moisture. At intervals, oöcysts were removed from the pellets by the flotation method described and examined for evidence of sporulation.

To determine the ability of oöcysts to sporulate inside feces dried in air, fresh pellets were placed in open dishes, stored at the desired temperatures, and the oöcysts were removed at intervals for observation by the flotation method.

In preparations in which little or no sporulation occurred, viability tests were conducted at intervals to determine the longevity of oöcysts under given conditions. The criterion for viability was the ability of the oöcysts to develop normal sporocysts after periods in the unsporulated condition, when the protoplasm is in the form of a spherical sporont. The viability test consisted in isolation of oöcysts in clean water on culture slides and storage for at least 3 days at room temperature. If no sporulation occurred within that time, the oöcysts were considered as dead, since maximum normal sporulation of oöcysts of *Eimeria arloingi* takes place within 48 hours after removal into clean water at room temperature.

In the results reported in this paper, oöcysts were classified as follows: Those in which there was no sign of protoplasmic segmentation, as oöcysts with undivided sporont; those containing four-lobed sporonts, four spheres, pyramids, or oval sporoblasts, as oöcysts with the intermediate stages; those in which the protoplasm had split into two to eight or even more fragments of unequal size, as oöcysts showing abnormal segmentation; and those containing four refractile equal-sized, roughly spindle-shaped sporocysts, as oöcysts with complete sporocysts.

The longevity of oöcysts in the sporulated condition obviously cannot be determined by the method described in this paper. The

criterion for viability of sporulated oöcysts is the production of infection in a susceptible host, which is beyond the scope of the present study. It is believed, however, that the conclusions derived from these viability experiments with unsporulated oöcysts can be applied as well to those in the sporulated state, with the advantage in favor of the latter as a result of an additional protective shell about each sporocyst.

SPORULATION AND VIABILITY OF OÖCYSTS OF EIMERIA ARLOINGI IN SHALLOW TAP-WATER CULTURES

Oöcysts in shallow tap-water cultures kept at near-freezing temperature (0°-5° C.) sporulated slowly, 2 to 3 weeks elapsing before a significant amount of sporulation was noted (table 1). At room temperature (20°-25°) segmentation was rapid and normal, maximum sporulation occurring within 48 hours. Since these cultures gave uniformly higher percentages of sporulation than any others in the series, the conditions were considered to be optimum for sporulation and became the basis for viability tests. Oöcysts in cultures kept at 32° segmented at an accelerated rate but in an abnormal manner. There were evidences of protoplasmic splitting in more than half of them at the end of 24 hours. At 72 hours, which is an interval sufficient for the production of four equal-sized spores in each oöcyst in cultures kept at room temperature, the protoplasm of most of the oöcysts had segmented into two to eight, occasionally more, fragments of unequal size and shape. There was no sign of sporulation in oöcysts kept at 40° C. for as long as 10 days.

TABLE 1.—Sporulation and viability of oöcysts of *Eimeria arloingi* beneath 2 mm. of clean tap water

Experiment No.	Temperature of preparation during sporulation test	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test					Results of viability test ¹																			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts showing abnormal segmentation	Oöcysts with complete sporocysts	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts																
1-----	° C.	0-5	{	1	Apr. 13, 1937	{	Days	No.	Pct.	Pct.	Pct.	Pct.	No.	Pct.	Pct.	Pct.													
																	7	40	100	0	0	0	18	-----	-----	-----			
																	13	111	59	23	0	0	-----	-----	-----	-----			
																	12	70	100	0	0	0	-----	-----	-----	-----			
																	16	66	76	21	0	3	-----	-----	-----	-----			
2-----	20-25	{	1	Apr. 19, 1937	{	Days	No.	Pct.	Pct.	Pct.	Pct.	No.	Pct.	Pct.	Pct.														
																23	56	7	63	0	30	-----	-----	-----	-----				
																5	694	4	0	0	96	-----	-----	-----	-----				
																2	May 27, 1937	{	Hrs.	26	121	50	24	0	26	-----	-----	-----	-----
3-----	32	1	Apr. 5, 1938	{	Days	No.	Pct.	Pct.	Pct.	Pct.	Pct.	No.	Pct.	Pct.	Pct.														
																30	95	19	38	0	43	-----	-----	-----	-----				
																48	88	3	3	0	94	-----	-----	-----	-----				
																24	108	47	7	26	20	-----	-----	-----	-----				
																41	107	18	7	54	21	-----	-----	-----	-----				
4-----	40	{	1	Mar. 30, 1938	{	Days	No.	Pct.	Pct.	Pct.	Pct.	No.	Pct.	Pct.	Pct.														
																1	100	100	0	0	0	79	34	63	3				
																2	Apr. 1, 1938	3	300	100	0	0	0	274	60	40	0		
																3	Apr. 5, 1938	10	100	100	0	0	0	100	100	0	0		

¹ Determined after 3 or more days beneath 2 mm. of clean water at 20°-25° C.

The results of the viability test, also given in table 1, showed that practically all the oöcysts kept at 40° C. in shallow tap-water cultures were killed by exposure for 2 days to that temperature and that all were killed in cultures exposed 3 and 10 days.

Failure to sporulate normally or at all at the higher temperatures is attributed to insufficiency of oxygen, since the oxygen-holding capacity of water diminishes with increase in temperature.

IN PUTREFYING FECAL SEDIMENT

At near-freezing temperature (0°–5° C.), all oöcysts in fecal sediment covered with shallow water remained unsporulated after 10 months' exposure (table 2); at room temperature (20°–25°), all were unsporulated after 1 year; and at 40°, there was no sporulation in 17 days. The protoplasm in these oöcysts retained the form of spherical sporonts, which gradually diminished in size and showed progressive disintegration as preparations aged.

TABLE 2.—*Sporulation and viability of oöcysts of Eimeria arloingi in fecal sediment covered with shallow water*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test		Results of viability test ¹			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts
	° C.			Days	Number	Percent	Number	Percent	Percent	Percent
1-----	0-5	1	May 26, 1937	50	100	100	200	4	0	96
				201	100	100	223	18	0	82
				314	200	100	123	19	62	19
				113	50	100	50	4	0	96
2-----	20-25	2	Sept. 22, 1937	368	100	100	100	0	100	0
				33	89	100	40	48	0	52
				127	100	100	38	100	0	0
				19	100	100	54	6	0	94
3-----	40	3	May 25, 1937	7	100	100	100	4	0	96
				1	212	100	156	100	0	0
				17	100	100	100	4	0	96
				4	100	100	46	78	22	0

¹ Determined after 3 or more days beneath 2 mm. of clean water at 20°–25° C.

The viability test showed the approximate point at which death of the protoplasm took place. Oöcysts kept in fecal sediment at near-freezing temperature lost viability slowly and gradually, as seen in table 2. There was no perceptible loss in ability to sporulate in nearly 4 months, after which oöcysts gradually died until only about one-fifth of them were viable at the end of 10 months. At room temperature the loss of viability was more rapid. Approximately half of the oöcysts remained viable at 1 month, whereas all were killed within 4 months. All oöcysts in preparations incubated at 40° C. were killed within 4 days.

The absence of sporulation in the oöcysts is attributed to the oxygen insufficiency resulting from putrefaction rather than to toxic products of the process. The fact that about 20 percent of these unsporulated oöcysts were still viable after 10 months at near-freezing temperature indicates that oöcysts may accumulate in wet situations in the outside environment and survive for many months during colder parts of the year and that they retain the ability to sporulate

and become infective to susceptible hosts when appropriate conditions are restored. This ability of oöcysts to live for long periods in wet situations in colder weather and the marked lethal influence of high temperatures suggest the advisability of selecting sunny, well-drained terrain for sheep lots and pasture and of eliminating sites that favor the accumulation of waste water.

INSIDE FECAL PELLETS IN WHICH NATURAL MOISTURE WAS PRESERVED

At near-freezing temperature (0° – 5° C.), oöcysts inside fecal pellets in which natural moisture was preserved sporulated slowly, only about one-fifth of them showing signs of segmentation within 10 months (table 3). Sporulation was rapid at room temperature (20° – 25°), only 3 days being required for the majority of the oöcysts to develop mature sporocysts. There was no sign of sporulation within 4 days in preparations incubated at 40° .

TABLE 3.—*Sporulation and viability of oöcysts of Eimeria arloingi inside fecal pellets in which natural moisture was preserved*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of sporulation test	Results of sporulation test				Results of viability test ¹			
					Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts with complete sporocysts	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with fragmented or disintegrated protoplasm	Oöcysts with normal sporocysts
					Num-ber	Per-cent	Per-cent	Per-cent	Num-ber	Per-cent	Per-cent	Per-cent
1.....	0-5	1	May 26, 1937	10	100	100	0	0	106	6	0	94
				126	117	86	12	2	100	7	0	93
				314	173	82	15	3	155	19	57	24
		2	Apr. 1, 1938	10	100	100	0	0	-----	-----	-----	-----
				3	577	29	11	.60	-----	-----	-----	-----
				9	744	20	0	.80	-----	-----	-----	-----
2.....	20-25	1	Apr. 19, 1937	36	103	3	0	.97	-----	-----	-----	-----
				1	163	92	8	0	-----	-----	-----	-----
				2	127	45	13	.42	-----	-----	-----	-----
		2	Mar. 31, 1938	4	109	46	4	.50	-----	-----	-----	-----
				3	98	4	1	.95	-----	-----	-----	-----
				7	100	5	0	.95	-----	-----	-----	-----
3.....	40	4	do	3	100	9	1	.90	-----	-----	-----	-----
				7	100	0	0	100	-----	-----	-----	-----
				4	100	100	0	0	100	98	2	0
				4	100	100	0	0	100	98	2	0

¹ Determined after 3 or more days beneath 2 mm. of clean water at 20° – 25° C.

The results of the viability test (table 3) showed that there was no appreciable loss of ability to sporulate in oöcysts inside natural pellets kept for 4 months at near freezing temperature, but during the next 6 months three-fourths of them lost viability. At 40° C., oöcysts inside the pellets were rapidly killed, being unable to sporulate after an exposure of 4 days.

These observations suggest the desirability of frequent changes of bedding straw and also of feeding from racks and elevated troughs rather than off the ground in order to eliminate environmental conditions favorable for the preservation of the natural moisture in fecal pellets and to reduce the chances of feed being contaminated with sporulated oöcysts.

INSIDE FECAL PELLETS DRIED IN AIR

Inside fecal pellets dried at room temperature (20°–25° C.), sporulation occurred before total desiccation. A majority of the oöcysts sporulated within 3 days (table 4) before the oöcyst walls were so extensively wrinkled and blackened that it was impossible to distinguish the contents. After 2 days of drying at 40°, oöcyst walls were extremely wrinkled, distorted, and blackened, but one-sixth of the oöcysts contained what appeared to be normal sporocysts.

TABLE 4.—*Sporulation of oöcysts of Eimeria arloingi inside fecal pellets dried in air*

Experiment No.	Temperature of preparation	Trial No.	Date of beginning of experiment	Duration of test	Oöcysts counted	Oöcysts with undivided sporont	Oöcysts with intermediate stages	Oöcysts in which wrinkling hid contents	Oöcysts with recognizable sporocysts
	° C.			Days	Number	Percent	Percent	Percent	Percent
1	20-25	1	Apr. 5, 1938	1	100	100	0	0	0
				2	93	3	5	38	54
				6	100	0	0	100	0
		2	Apr. 11, 1938	3	67	10	0	10	80
				7	54	0	0	93	7
2	40	1	Apr. 5, 1938	2	104	39	2	43	16
				6	100	0	0	100	0

The data of these experiments indicate that drying is probably fatal to oöcysts within several days or a few weeks as a result of the loss of water necessary for the vital activities of protoplasm. This observation again emphasized the importance of dry, well-drained land for sheep lots and pasture.

SUMMARY

The results of sporulation and viability experiments on oöcysts of *Eimeria arloingi* from domestic sheep are presented. These experiments demonstrated several facts of practical importance in the control of ovine coccidiosis.

Oöcysts covered with 2 mm. of clean water sporulated slowly at near-freezing temperature (0°–5° C.) and normally and rapidly at room temperature (20°–25°). At 32° sporulation was accelerated but segmentation was abnormal, and at 40° the oöcysts failed to show signs of sporulation. The viability test showed that the unsporulated oöcysts in the cultures incubated at 40° were killed when exposed for 3 days. Abnormal segmentation and failure to sporulate at the higher temperatures were apparently due to insufficiency of oxygen.

There was no indication of sporulation at near-freezing, room, or incubator temperatures in oöcysts kept in fecal sediment covered with a shallow layer of water. Failure to sporulate under these conditions was attributed to lack of oxygen resulting from putrefaction. About 20 percent of the unsporulated oöcysts were still viable after 10 months at near-freezing temperature, indicating that oöcysts may accumulate in wet situations in the outside environment and survive for many months during the colder parts of the year, and that they retain the ability to sporulate and become infective to susceptible hosts when appropriate conditions are restored.

Oöcysts inside fecal pellets in which moisture was preserved showed little sporulation at near-freezing temperature, abundant and rapid sporulation at room temperature, and failure to sporulate at 40° C. These observations indicate that simple, sanitary measures in lamb raising, such as frequent changes of bedding straw and feeding from elevated racks and troughs, would greatly reduce the chances of contamination of the feed with resultant infection.

When fecal pellets were dried in air at room temperature and at 40° C., many of the contained oöcysts sporulated before desiccation had produced such extensive wrinkling and shrinkage that recognition of contents was impossible. It was concluded that drying was probably fatal to oöcysts within several days or a few weeks, emphasizing the importance of dry, well-drained land for sheep lots and pasture.

THE NATURE OF GROWTH SUBSTANCE ORIGINATING IN CROWN GALL TISSUE¹

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INTRODUCTION

The presence of growth substance in more than the normal concentration (10)² has been demonstrated in cultures of the crown gall organism, *Phytoplasma tumefaciens* (Smith and Town.) Bergey et al. (1, 2, 9, 10, 11) and also in recently inoculated gall tissue. The growth substances obtained from cultures of micro-organisms have been identified in a number of instances as beta-indole-acetic acid (1, 7, 9, 14). Early reports identified growth substances from the higher plants as auxin *a* and *b* (4, 5, 6). More recently the presence of beta-indole-acetic acid in higher plants has been reported (8). It is desirable to know the nature of the growth substance in crown gall for various reasons; e. g., should the growth substance normally produced by the host plant prove to be different from that produced by the crown gall organism in culture, it would be possible to determine whether the growth substance present in the gall tissue is produced by the bacteria or by the host cells. Consequently, the identification of the growth substances obtained from tomato gall tissue, from tomato foliage, and from crown gall culture was undertaken.

METHODS

The method chosen was that of Kögl and his associates (6). Beta-indole-acetic acid is stable in basic solution and unstable in acid solution; auxin *a* is stable in acid solution and unstable in basic solution, and auxin *b* is unstable in both acid and basic solution. Thus, it is possible to classify these growth substances on this basis.

The gall tissue was obtained from inoculations on stems of tomato (*Lycopersicon esculentum* Mill.). Before inoculation the plants were decapitated and the leaves and axillary buds were removed from the stems as far down as the inoculations extended. This was done to prevent the growth substance that is normally produced in these parts from entering the gall tissue. Six weeks after inoculation the galls were harvested and prepared for study. Some were extracted fresh and some were frozen and dried while frozen in the lyophile apparatus (3). An ether extract made of denuded, uninoculated stems indicated that growth substance was present in a concentration equal to 0.007 gamma of beta-indole-acetic acid per denuded stem weighing 0.4 gm. This is about one-tenth as much as was found in parallel material from inoculated stems which, however, weighed about 4 gm. Thus, the growth substance present in 1 gm.

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² Italic numbers in parentheses refer to Literature Cited, p. 538.

(wet weight) of tissue was not very different in either case, being about 0.0018 gamma. Doubtless higher yields of growth substance can be secured with improvements in methods of extraction.

Growth substance from the above-described material, from tomato foliage, from crown gall culture, and from corn meal, as well as beta-indole-acetic acid, were all tested for stability toward acid and base. Beta-indole-acetic acid and corn-meal extract (auxin *a* and *b*) were included as checks on the method. The tomato foliage and culture extracts were included for comparison with the gall extract.

Fresh tissue for growth substance was extracted according to Van Overbeek's method (12). One kilogram of fresh sliced tissue was immersed in 2,000 cc. of peroxide-free ether and left overnight at 4° C. The following morning the ether extract was placed on a steam bath. The residues in this case and in others mentioned below were taken up in 5 cc. of distilled water. Dried tissue and corn meal were similarly extracted at the rate of 50 gm. to 500 cc. of ether. Extractions of crown gall cultures grown in peptone broth were made 3 months after seeding with 500 cc. of ether to each 250 cc. of culture.

Following, as far as practicable, the procedure of Kögl et al., the sensitivity toward acid and base was tested. One cubic centimeter of growth-substance solution was placed in each of three vials. To the first was added $\frac{1}{2}$ cc. N/2 HCl; to the second, $\frac{1}{2}$ cc. N/2 KOH; and to the third $\frac{1}{2}$ cc. distilled water and one drop of saturated phosphate buffer (pH 7.0). The three vials were loosely covered and steamed for 3 hours in the Arnold steamer. After removal from the steamer they were neutralized. One drop of saturated phosphate buffer (pH 7.0) was then added to vials 1 and 2. They were then tested for the presence of active growth substance. One cubic centimeter of untreated growth substance solution was diluted with 1 cc. of distilled water (pH 7.0) and tested along with the treated samples.

Growth substance was estimated by means of Went's standard technique (15) as discussed earlier (11). The growth-substance concentration of each preparation was expressed as the average negative curvature produced in 12 coleoptiles.

EXPERIMENTAL RESULTS

The results are summarized in tables 1 and 2. These are based on 12 trials involving 6,912 *Avena* coleoptiles. Not reported are results of 8 preliminary trials involving 576 coleoptiles. These trials were made in the course of determining the most usable concentration of acid and base and the most desirable time of treatment. While these results were in complete agreement with those reported, they were omitted for the sake of uniformity and brevity.

The growth substances from the gall tissue, tomato foliage, and crown gall culture were all much more stable in basic solution than in acid solution. This suggests that some substance comparable in certain reactions to beta-indole-acetic acid was present in the ether extracts of these materials. These same preparations showed some reduction in activity below that of the untreated samples, which might have been slightly greater than indicated here, because the untreated samples gave curvatures beyond the maximum quantitative angle. However, other trials within the quantitative range indicate that this reduction has little if any effect on the conclusions drawn. Following treatment in basic solution, there was much less or no reduction in

activity below that of the sample treated in neutral solution. Consequently, there is little or no evidence in these data that any growth substance unrelated to beta-indole-acetic acid is involved in the crown gall material studied.

TABLE 1.—Average negative curvatures of *Avena coleoptiles* obtained with growth substances from crown gall and several other sources before and following heat treatments in acid, basic, and neutral solutions¹

Source of growth substance	Average negative curvature of <i>Avena coleoptiles</i> following the treatments indicated			
	Acid	Basic	Neutral	Untreated
	Degrees	Degrees	Degrees	Degrees
Beta-indole-acetic acid.....	4	15	20	24
Do.....	2	12	20	25
Corn meal.....	18	13	24	23
Do.....	20	12	20	24
Fresh tomato gall tissue.....	3	16	12	20
Do.....	1	15	16	16
Lyophilized tomato gall tissue.....	0	18	21	18
Do.....	1	11	13	13
Fresh tomato foliage.....	7	17	19	24
Do.....	2	13	11	21
Crown gall culture.....	12	24	20	26
Do.....	1	15	8	13

¹ Comparison can be made horizontally, between treatments of the same material, but should not be made vertically between different materials.

TABLE 2.—Reduction in activity of growth substances from crown gall tissue and several other sources following heat treatment in acid, basic, and neutral solution

Source of growth substance	Reduction in activity following treatments indicated in percentage of untreated sample		
	Acid	Basic	Neutral
	Percent	Percent	Percent
Beta-indole-acetic acid.....	88	45	15
Corn meal.....	20	45	7
Fresh tomato gall tissue.....	88	11	20
Lyophilized tomato gall tissue.....	94	6	0
Fresh tomato foliage.....	81	32	32
Crown gall culture.....	72	3	31

The method distinguished between beta-indole-acetic acid and auxins *a* and *b* in trials made with beta-indole-acetic acid and an ether extract of corn meal. The indolic acid was much less stable in acid solution than in basic solution, although in the basic solution there was some reduction in activity below that in neutral solution. The auxin was highly stable in neutral solution, but was only moderately stable in acid solution and somewhat less so in basic solution. This suggests the presence of both auxin *a* and auxin *b*, the figures obtained in these trials indicating a mixture of about equal amounts of the two types. The activity following basic treatment is attributed to incomplete inactivation of auxin *b* rather than to the presence of beta-indole-acetic acid because the sum of the curvatures obtained after acid and after basic treatment far exceeds the curvature obtained with the untreated sample.

The growth substance obtained from the foliage of the normal plant proved to be indistinguishable, on the basis of these tests, from that in the crown gall culture. Thus it is not possible at present to conclude whether the bacteria or the host cells supply the growth substance in crown gall tissue. While these tests with crude extracts are not nearly so reliable as diffusion experiments might be, they suggest (1) that the active substance in gall and stem are similar; and (2) that this substance resembles beta-indole-acetic acid in its sensitivity to acid and alkali. Lefèvre (8) determined the phenyl or indole compounds in expressed juice, but these may not be active in causing growth.

While it appears that the same type of growth substance occurs in crown gall culture and crown gall tissue, it seems wise to reserve judgment about beta-indole-acetic acid being the major cause of gall formation. Among the reasons are: (1) The amount of growth substance detected in crown gall tissue is a very small fraction of that required to produce a similar proliferation with artificial application (10); (2) substances diffusing from inoculations made with virulent crown gall bacteria stimulate development of galls by inoculation with attenuated crown gall bacteria, while even strong applications of beta-indole-acetic acid do not (10); and (3) responses of a number of plants to crown gall bacteria do not parallel their reactions to applications of beta-indole-acetic acid (13).

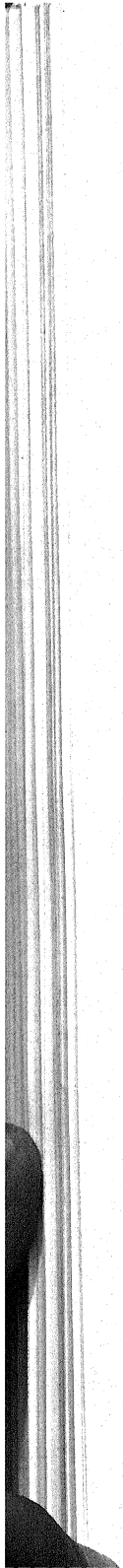
SUMMARY

Growth substances obtained by ether extraction from tomato crown gall tissue, tomato foliage, and crown gall culture on peptone broth all appeared to contain either beta-indole-acetic acid or material similar in its sensitivity to acid and alkali. Identification was based on the stability of a crude extract in hot acid and basic solutions. No evidence was obtained of the presence of auxin *a* or *b* in these extracts. The growth substance measured might have come either from the plant or from the bacteria.

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INFLUENCE OF BORON ON FLOWER-BUD DEVELOPMENT IN COTTON¹

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INTRODUCTION

Although the role of boron in plant nutrition has received a great deal of attention in the past few years most investigators have emphasized the relation of this element to the vegetative growth of plants or to imperfections in the fruit. Brenchley and Warrington,² Johnston and Fisher,³ Shive,⁴ Eaton,⁵ and others have called attention to the influence of boron on fruiting but have offered no evidence to show that any particular phase of the fruiting cycle was specifically concerned.

The results reported in this paper emphasize the phase of the reproductive cycle of cotton in which the boron supply may be a limiting factor.

MATERIALS AND METHODS

Jars and special covers of pyrex glass used in other water-culture studies⁶ were employed in these experiments. Investigators in this field have generally avoided borosilicate glass, but experience has shown that pyrex glass does not supply sufficient boron to the young cotton seedlings in midsummer to protect them from severe boron deficiency even in the first week of growth (fig. 1).

Salts for the nutrient solutions were of the same lot used in other trace-element studies and for the 1937 cultures chemically pure salts were recrystallized three times from water redistilled from pyrex stills. For the 1938 cultures two additional recrystallizations were made from water double-distilled from pyrex stills.

The basal solution had the following composition:

Ca (NO ₃) ₂	Mole 0. 0030
MgSO ₄ 0020
KH ₂ PO ₄ 0010

To all cultures was added 0.1 p. p. m. Mn as MnSO₄·2H₂O. Iron as ferric tartrate was added as needed. Manganese and boron were the only so-called trace elements added to the culture solutions in 1937 or at the beginning of the 1938 work. However, the 1937 plant leaves never had a satisfactory green color and after the same faint and persistent chlorosis developed in the 1938 cultures, copper as

¹ Received for publication March 24, 1939, Paper No. 62 of the Journal Series of the Georgia Experiment Station.

² BRENCHLEY, WINIFRED E., and WARRINGTON, KATHERINE. THE ROLE OF BORON IN THE GROWTH OF PLANTS. *Ann. Bot.* [London] 41: 167-187, illus. 1927.

³ JOHNSTON, EARL S., and FISHER, PAUL L. THE ESSENTIAL NATURE OF BORON TO THE GROWTH AND FRUITING OF THE TOMATO. *Plant Physiol.* 5: 387-392, illus. 1930.

⁴ SHIVE, JOHN W. THE ADEQUACY OF THE BORON AND MANGANESE CONTENT OF NATURAL NITRATE OF SODA TO SUPPORT PLANT GROWTH IN SAND CULTURE. *N. J. Agr. Expt. Sta. Bul.* 603, 36 pp., illus. 1936.

⁵ EATON, FRANK M. BORON REQUIREMENTS OF COTTON. *Soil Sci.* 34: 301-305. 1932.

⁶ GEORGIA EXPERIMENT STATION. MANGANESE REQUIREMENTS OF COTTON. *Ga. Expt. Sta. Ann. Rpt.* (1937-38) 50: 62-63. 1938.

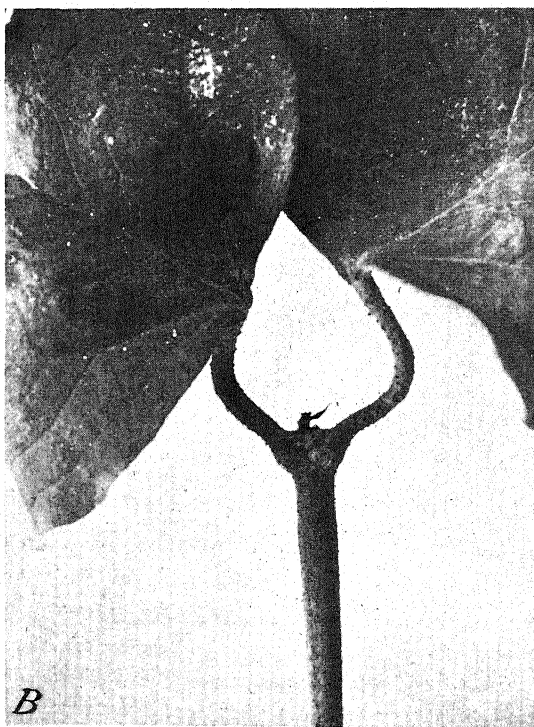
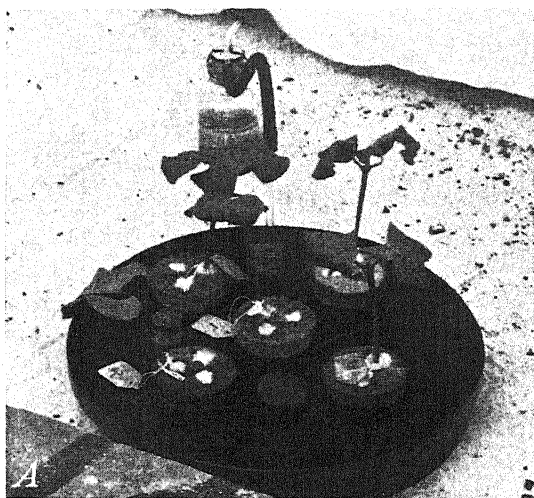


FIGURE 1.—A, Cotton seedlings after growing 37 days in a nutrient solution, without added boron, in a pyrex jar; B, plant from group in A showing failure of terminal bud development.

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and zinc as ZnCl_2 at the rate of 0.01 p. p. m. and 0.1 p. p. m., respectively, were added to each jar on July 20 and at each renewal thereafter. The color of the leaves of these plants after these additions was satisfactory. Boron was added as boric acid. The stock solutions and distilled water used in preparing these nutrients were stored and handled in pyrex glass.

The solutions were renewed June 29, July 16, August 5, 19, 27, 1937, and June 15, 27, July 14, 25, and August 8, 19, 1938. In the later stages of growth, the nitrogen was almost completely absorbed within 3 or 4 days after the solutions were renewed, and consequently growth was limited by insufficient nutrient supply.

The delinted seed of the Durango variety which was used in these cultures contained 23 p. p. m. of boron or about 3 gamma per seed. Fifteen seedlings were started in each jar and they were thinned to five after about 2 weeks and to two after about 5 weeks. After the beginning of the fruiting stage in 1937, the flower buds, or squares, were tagged with white tags and counted once each week. However, in the late-growth stages it was found that many buds were formed and shed within 1 week and that no accurate record of total buds formed could be obtained for the 1937 crop. In the 1938 studies

the buds were tagged and counted twice each week. Every morning white tags were removed from newly opened blooms and replaced by colored tags. In the 1938 cultures the colored tags were dated.

EXPERIMENTAL DATA

In the 1937 cultures there was no noticeable difference in the general appearance of the plants at the different boron levels for the first 8 or 9 weeks. Abscission of flower buds and scarcity of blooms, however, were noticeable within the first 9 weeks on plants at the 0.1-p. p. m. boron level. As the growing period advanced, the young buds were shed when they were much smaller and, finally, there was some irregularity in leaf development in the growing tops of these plants, indicating a deficiency of boron for the young growing points. At this stage the young flower buds blackened and died in a manner comparable to that seen in terminal buds in cases of severe boron deficiency.

TABLE 1.—*Influence of boron supply upon growth and flowering of cotton planted June 15 and harvested Sept. 11, 1937*

[Average of four plants]

Boron added (p. p. m.)	Height of plants	Green weight of plants	Flower buds Sept. 11	Blooms
	<i>Centimeters</i>	<i>Grams</i>	<i>Number</i>	<i>Number</i>
0.1.....	163.7	1,076.0	53.1	10.7
1.0.....	172.5	1,257.0	46.7	53.2
5.0.....	148.7	1,199.0	29.7	45

As the data of table 1 show, there was no decided difference in the vegetative growth of the plants at the different boron levels, but the 0.1-p. p. m. boron plants produced very few blooms. Most of the green-weight difference was due to the young bolls on the plants at the higher boron levels.

The experiment was repeated on a larger scale in 1938. Again there was little difference in the appearance of the plants at the two boron levels for the first 8 weeks. The flower buds on the plants at the lower boron level developed to a fair size and then most of them became chlorotic, the bracts flared open, and they dropped. As the growing period advanced, the flower buds abscised at smaller sizes so that during the last 10 days of the study they darkened and dropped when they were so small that they were scarcely recognizable, and it is probable that the recorded figure for total flower buds for this series is low in spite of the fact that they were tagged twice each week. About August 15 the branches near the tops of this group of plants became very brittle, developed short internodes, and the leaf buds tended to darken and show other irregularities in development usually associated with boron deficiency. This development is illustrated in figure 2. As table 2 shows, the results are in general agreement with those of 1937.

In this case the mean difference in flowers per plant, 40.4, with a pooled standard deviation of 10, is highly significant.



FIGURE 2.—Low-boron plant (0.1-p. p. m. group, Table 2), August 28, 1938, showing irregularity of leaf development near top.

TABLE 2.—*Influence of boron supply upon growth and flowering of cotton planted June 3 and harvested Aug. 28, 1938*

[Average of 14 plants]

Boron added (p. p. m.)	Height of plants	Green weight of plants	Flower buds Aug. 28	Flower buds shed	Total flower buds	Blooms
	<i>Centimeters</i>	<i>Grams</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>
0.1.....	155.1	1,149.0	54.6	98	162.9	10.2
1.0.....	182.0	1,303.0	62.1	40	153.2	50.6

DISCUSSION

Since the nutrient concentration was not held constant, these results give little indication of the optimum concentration of boron for growth and fruiting of cotton in water culture. They do emphasize, however, the importance of boron in the flowering of cotton and indicate that an insufficiency of this element may be the cause of unfruitfulness. Although approximately 70 percent of the flowers of the low-boron plants, 1938 series, appeared by August 10, before boron deficiency had become manifest in the vegetative parts of the plants, the abscission of young buds from this group of plants and from those at the same level the previous year was apparently abnormal in the early stages. This observation along with the fact that vegetative growth was not severely checked at any stage in the low-boron series suggests that the concentration of this element necessary for flower-bud development in cotton is higher than that required by the vegetative parts. But the blackening of young flower buds in a manner quite similar to that observed in young leaf buds in cases of severe boron deficiency also suggests that the specific effects of the deficiency upon the tissues involved are probably the same.

From the records of total flower buds per plant (table 2) it may be seen that the boron level which was too low for flower-bud development had no apparent effect on flower-bud initiation.

SUMMARY

Water-culture studies of the boron requirements of cotton brought out the fact that this element is necessary for flower-bud development in this plant, and that flowering may be seriously limited by a supply of boron that is sufficient for fair vegetative growth.

There is no evidence from these results that boron has any relation to flower-bud initiation in cotton.

PHOTOSYNTHETIC STUDIES OF MUTATIONAL BARRENNESS IN THE MONTMORENCY CHERRY¹

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INTRODUCTION

The reality of bud mutation by means of which unique plant forms arise is no longer seriously doubted. That the mutant's peculiar, not to say abnormal, behavior has a discoverable physiological basis seems altogether likely. And this, be it said, is not to rule out genetics, since no objection need be made to the notion of an underlying genetic condition, enforcing and finding its expression through altered yet controlled physiological processes.

A case in point is that of a bud mutant showing the permanent characteristic of not forming fruit buds and of being always barren. Investigations have shown that an accumulation of carbohydrates in the spurs of spur-bearing trees is necessary for fruit-bud differentiation. The rate of carbohydrate synthesis must exceed the rate of utilization of the carbohydrates in vegetative growth if there is to be an accumulation in the tissues of the spurs. Possibly the defect of this antecedent condition—a rate of photosynthetic activity that is relatively too low—characterizes the perpetually barren mutant. To test the validity of this hypothesis a series of experimental observations was made on a mutant in the Montmorency cherry (*Prunus cerasus* L.).

TREES USED IN THE TESTS

In the beginning, a limb mutant was detected and marked in an orchard of Montmorency trees near South Haven, Mich. The limb was conspicuous for its lack of fruit-bud formation and consequent barrenness. During the season of 1925, after several years of this behavior, buds were taken from it and propagated on mahaleb stock. Six of the trees thus obtained were selected in 1929 and transferred from the nursery to an orchard plot at the Graham Horticultural Experiment Station, Grand Rapids, Mich. The trees were planted in a row, the usual distance apart, and thereafter were given ordinary attention and culture. Upon coming to bearing age, they exhibited the following characteristics, which have remained constant:

- Tree 1. Barren, except two main limbs that are normal in fruitfulness.
- Tree 2. Wholly barren.
- Tree 3. Barren, except one main limb that is somewhat productive.
- Tree 4. Like tree 1, except that only one limb is fruitful.
- Tree 5. Wholly barren.
- Tree 6. Entirely normal in bearing habit and productivity.

In 1934, 5 years after the trees were planted, experiments were begun to determine whether or not the mutational barrenness arose from inadequate synthesis of carbohydrates and a deficiency of their accumulation in the spurs.

¹ Received for publication September 24, 1938. Journal article No. 233 (n. s.), Michigan Agricultural Experiment Station.

ANALYSIS OF SPURS

Samples of spurs were taken at intervals between June 6 and August 3, 1934. Tree 5 (barren) and tree 6 (normal) were used for comparison. Sampling was restricted to the nonbearing spurs of lateral branches on the wood of the season before, and was randomized by the selection of laterals generally around and over the tree.

The sample, consisting of 125 to 200 spurs, was heated immediately at 90° C. for 40 minutes. It was then transferred to an oven held at 67° C. and brought to dryness. Its preparation for analysis was completed by grinding to pass a 60-mesh sieve. Later, in actual readiness for analysis, drying was accomplished at 70° C.

Free reducing substances, starch, and polysaccharides other than starch, were determined. The analytic procedure for each of these was the customary one, involving the Shaffer-Hartmann and Quisumbing-Thomas routine. The results are shown in figure 1.

At Grand Rapids detectable fruit-bud differentiation in the Montmorency cherry begins within the last 2 weeks of July. Doubtless this observable stage is preceded by a period of incipient preparation, the beginning and length of which, if knowable at all, has not been determined. Figure 1 shows that fruit-bud differentiation was preceded by an emphatic rise in free-reducing substances in the spurs, followed by a steep and prolonged decline. A second upward tendency coincides with its visible inception and continues during its progress.

The graphs for polysaccharides other than starch are the inverted image of those for free-reducing substances. As to starch, the content increases continuously, from the beginning for the normal tree and after June 21 for the barren (mutant) tree.

A general comparison of the two trees shows that the normal one runs higher in free-reducing substances and starch and lower in polysaccharides other than starch. This indicates both the maintenance of a more abundant supply of elemental carbohydrates in the normal tree and their sustained accumulation, in the form of a higher content of starch, in its spurs. The latter finding is the more remarkable because the tree supported a full crop of fruit until the first week in July, the time of harvest.

Amino nitrogen was determined for the same samples, the Van Slyke apparatus and technique being employed for its estimation. The determinations are presented graphically in figure 2.

Amino acids, the simpler elements for the synthesis of proteins, increase in the spurs in the early part of the season, and then decline (fig. 2). The falling off starts later in the barren tree (July 6) than in the normal. Thereafter it is continuous in this tree, whereas in the normal tree it ceases about midway in the seasonal range, and thenceforward, just before and through the period of fruit-bud formation, holds at this higher level.

All in all, the results suggest a relatively low rate for photosynthetic activity, and for the production and accumulation of starch, both prior and subsequent to fruit-bud formation, in tree 5, the strictly mutational tree. However, the significance of such analyses, especially for samples infrequently taken, should not be given too much weight. Their bearing upon the rate of photosynthetic activity and manufacture is not as close as is required for conclusive interpretation, and

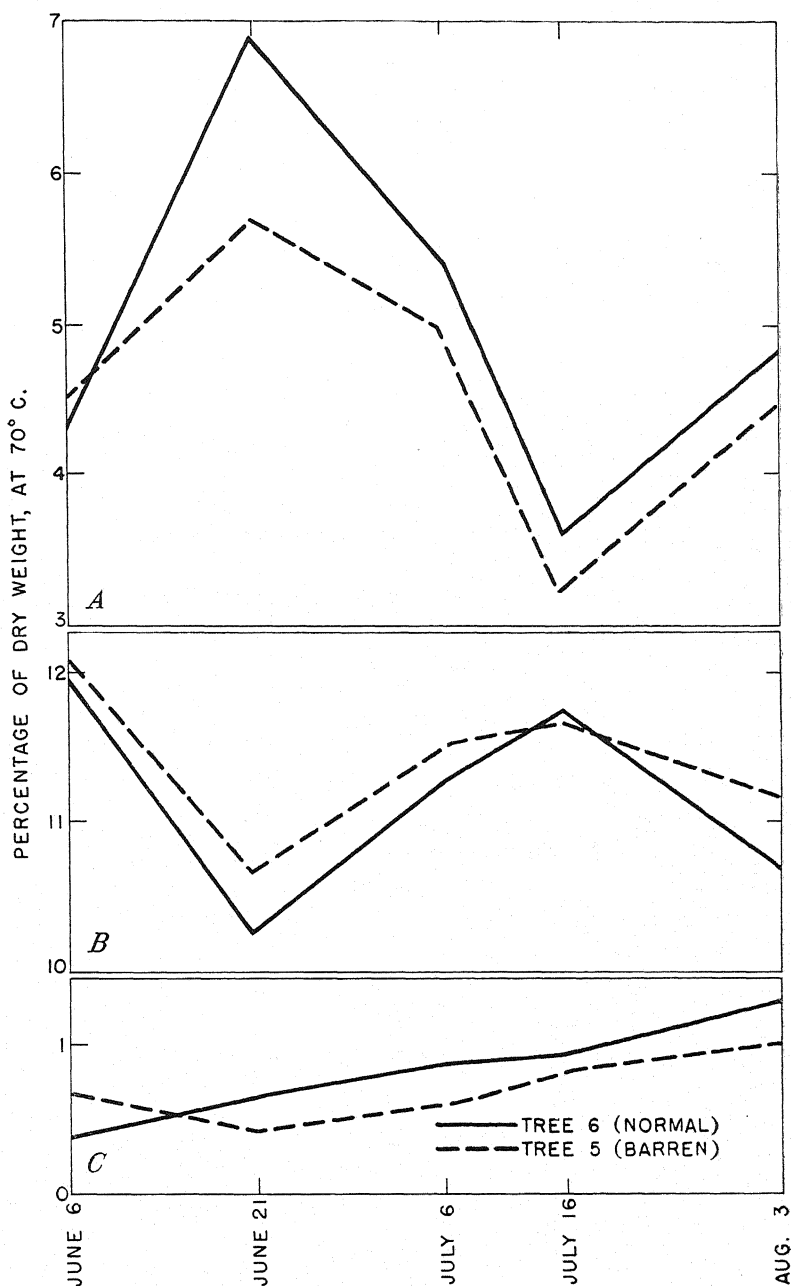


FIGURE 1.—Free reducing substances (A), polysaccharides other than starch (B), and starch (C) in spurs from a barren and from a normal tree between June 6 and August 3, 1934.

they should be checked by experimental procedures that are more direct and at the same time better adapted for securing data essential to the calculation of a rate for a physiological process.

PHOTOSYNTHESIS STUDIES

Photosynthesis determinations were made during the season of 1936, usually at regular intervals, on samples taken every 2 hours on clear days during a period of 24 hours.

The samples were obtained by the leaf-punch method, devised by Ganong. When refined by the familiar corrections for translocation and respiration, and changes in the weight of ash, as it was in this work, this method is satisfactory for use in the orchard and for taking many samples at random over a tree or any large part of a tree.

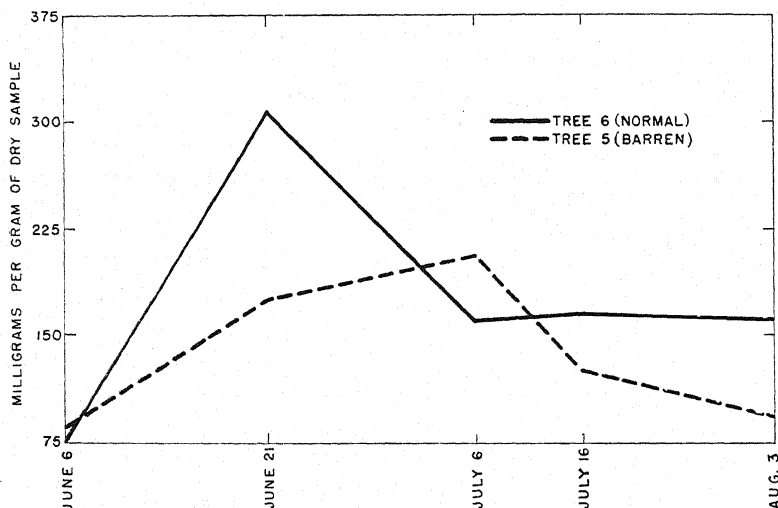


FIGURE 2.—Amino nitrogen in spurs taken from a barren and from a normal tree between June 6 and August 3, 1934.

Each sample consisted of 30 disks—one each from the leaves of spurs in the middle sections of laterals taken generally over the tree or the portion of the tree involved in the comparison. The Ganong leaf punch cuts a disk 1 cm.² in area. Hence, a sample was equivalent to 30 cm.² of leaf surface.

The trees were cultured and sprayed alike. The spray material, when present, was wiped from the leaves selected for use, at the beginning of the 24-hour sampling period. Its uneven distribution on the leaves necessitated this precaution.

The first three samplings (shown in table 1) were with leaves of the spurs of laterals on 1934 wood. In the first two of these three samplings fruiting spurs were chosen on tree 6 and on the fruitful parts of trees 1 and 3 for comparison with barren spurs on tree 5 and the barren parts of trees 1 and 3. Thereafter, beginning July 25, nonfruitful spurs were selected exclusively throughout, these on the laterals of 1935 wood. The results are given in table 1.

In table 1, the ten 24-hour series are divided in half by the first row of totals and corresponding rates. The upper half, July 8-9 to August 8-9, covers approximately the period from the picking of the fruit, when present, to the conclusion of the process of fruit-bud formation. The lower half, August 15-16 to September 17-18, covers the time from fruit-bud formation to the onset of senility in the leaves.

TABLE 1.—*Amount and rate of photosynthate production for spur leaves from barren and fruitful trees or parts of trees, 1936*

Date	Total photosynthate in 24 hours per square meter of leaf area				Photosynthate produced per hour per square meter of leaf area			
	Trees 1 and 3		Tree 5 (barren)	Tree 6 (normal)	Trees 1 and 3		Tree 5 (barren)	Tree 6 (normal)
	Barren parts	Fruitful limbs			Barren parts	Fruitful limbs		
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
July 8-9.....	-3.133	-2.533	-3.633	+1.667	-0.131	-0.106	-0.151	+0.069
July 17-19.....	-2.633	+2.700	-1.699	+366	-.110	+.113	-.071	+.015
July 25-26.....	+3.799	+9.332	-.167	+2.833	+.158	+.389	-.007	+.118
Aug. 1-2.....	-5.099	+1.367	-2.933	-4.633	-.213	+.057	-.122	-.193
Aug. 8-9.....	-.933	-2.566	+1.467	+5.900	-.039	-.107	+.061	+.246
Total.....	-7.999	+8.300	-6.965	+6.133	-.067	+.069	-.058	+.051
Aug. 15-16.....	-.967	-6.767	+2.100	+.467	-.040	-.282	+.087	+.020
Aug. 22-23.....	+1.933	+1.567	+8.133	-1.733	+.081	+.065	+.339	-.072
Aug. 29-30.....	+3.000	+1.000	+2.300	+.483	+.125	+.042	+.096	+.020
Sept. 6-7.....	+4.700	+.867	+6.533	+1.700	+.196	+.036	+.272	+.071
Sept. 17-18.....	+9.900	-2.167	+2.200	+7.600	+.413	-.090	+.092	+.317
Total.....	+18.566	-5.500	+21.266	+8.517	+.155	-.045	+.177	+.071
Grand total.....	+10.567	+2.800	+14.301	+14.650	+.044	+.012	+.060	+.061

During the first of the two periods, the apparent rates of photosynthetic activity for the normal tree and normal parts of trees are higher. The rates for the period as a whole are as +0.051 to -0.058, and as +0.069 to -0.067. The differences in total net production of photosynthate are wide, being +6.133 gm. for tree 6 as compared with -6.965 for tree 5, and +8.300 gm. for normal limbs of trees 1 and 3 as compared with -7.999 for the barren portions. The change, of the nature of a reversal, for the second period is worthy of note. The barren tree (5) and the barren parts of the mixed trees (1 and 3) have higher rates and sustain these to the end, which results in much greater amounts of photosynthate. (Note the second line of totals and rates.)

Table 1 (bottom line) gives the totals and rates for the two periods combined, or for the entire season. The values for the barren and normal trees are practically identical, but the values for the barren parts of trees 1 and 3 are very much higher than those for the fruitful limbs. Whether or not this difference, which does not appear in the comparison of the wholly normal and wholly barren trees, is peculiar to trees that are mixtures, through partial reversion, of barrenness and fruitfulness, is a question worthy of interest and attention.

It may be inferred, at least tentatively, that barren mutants in the Montmorency cherry are, as compared with the normal form, photosynthetically deficient during the period of fruit-bud initiation, differ-

entiation, and early development, but not so with respect to the later part of the season or the season as a whole.

VEGETATIVE GROWTH MEASUREMENTS

In a study of the vegetative development of the trees made in 1937, increase in the diameter of the twig, on the wood of the previous year, was taken as the criterion for growth. The twigs to be measured were chosen over the tree, or part of the tree, on May 5. These were permanently marked on this date of selection and first measurement (by means of a caliper), and thereafter remeasured at designated times during the remainder of the season.

Certain branches on tree 6, and also on the normal fruiting branches of tree 3, were deflorated immediately after flowering. This relieved the trees of the burden of setting and developing fruit and was expected to cause their approach to the barren forms in the magnitude of vegetative growth as determined by increase in diameter of twigs. The results are given in table 2.

TABLE 2.—*Growth in diameter of twigs on barren, fruitful, and deflorated trees, 1937*

Tree No.	Location of twigs on branches	Twigs	Fruit	Average diameter of twigs on—										Gain
				May 5	May 22	June 14	July 7	July 22	Aug. 2	Aug. 12	Aug. 23	Sept. 1	Sept. 13	
				Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	Mm.	
1	{ Barren	No. 63	Lb. 36	3.6	3.6	4.3	5.0	5.2	5.3	5.4	5.4	5.5	5.5	53
	{ Fruitful	82		2.9	2.9	3.3	3.7	3.9	4.0	4.0	4.2	4.2	4.2	45
3	{ Barren	88		3.1	3.3	3.9	4.4	4.9	5.0	5.1	5.2	5.3	5.3	71
	{ Deflorated	55		2.8	2.9	3.3	3.8	4.1	4.2	4.3	4.4	4.5	4.5	61
5	{ Fruitful	55	12	2.7	2.9	3.2	3.5	3.8	3.8	3.9	4.0	4.1	4.1	52
	{ Barren	85		3.3	3.5	4.3	5.0	5.4	5.5	5.6	5.8	5.9	5.9	79
6	{ Fruitful	51	120	3.0	3.2	3.5	3.8	4.1	4.2	4.2	4.3	4.5	4.5	50
	{ Deflorated	66		3.0	3.1	3.7	4.3	4.7	4.8	4.8	5.0	5.1	5.3	77

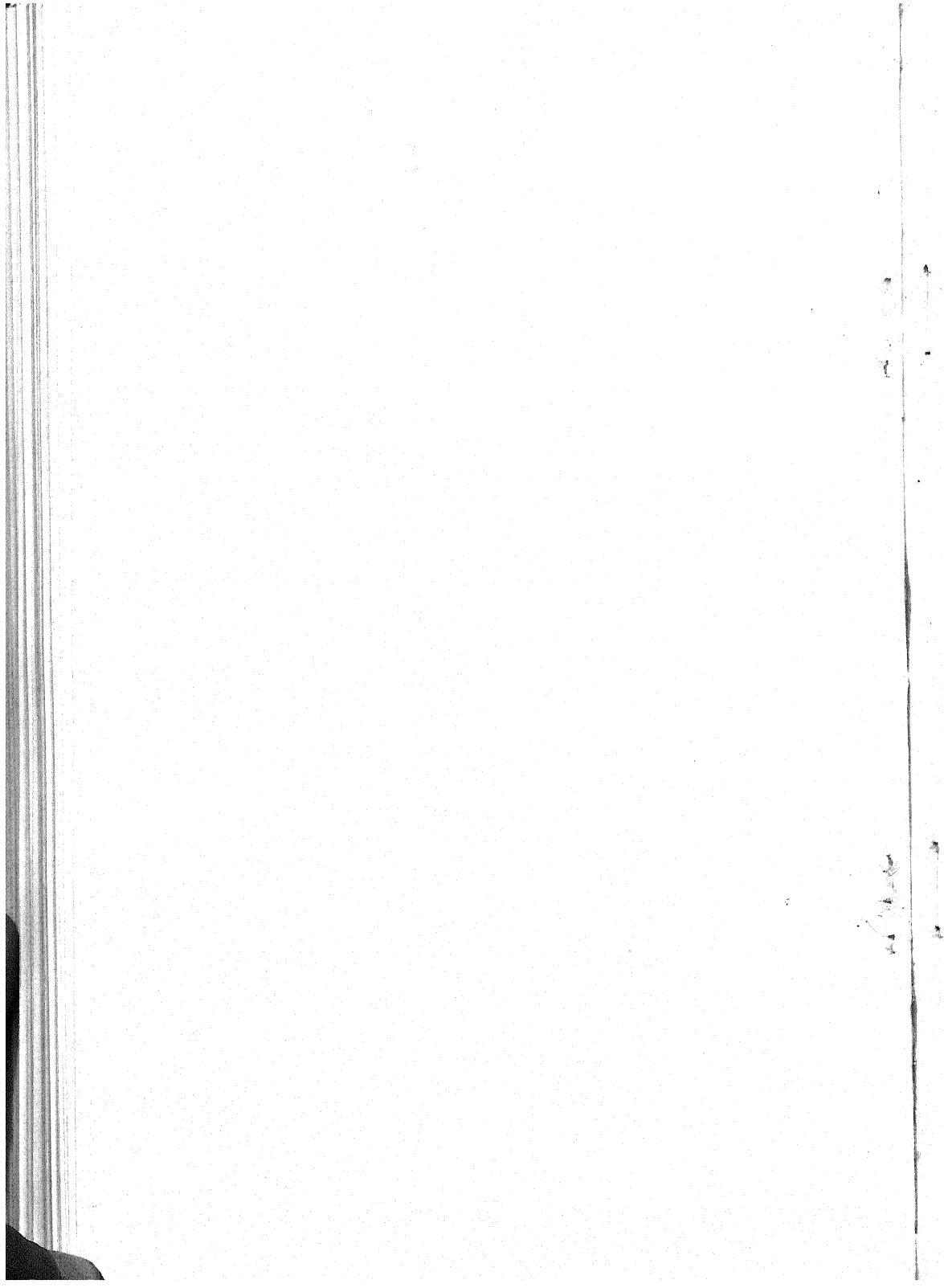
¹ For experimental branches; for entire tree, 88 pounds.

The data in table 2 afford no surprises. The relative gains in purely vegetative growth of the barren forms for the entire season exceeded those of the normal by significant differences. The latter, of course, sustained the additional outlay of manufactured materials required for the processes of blooming, fruit development, and fruit-bud formation. Where this drain was checked by defloration (in trees 3 and 6), and this supply released from the usual channel of fruit development, the twigs on the deflorated branches responded by making gains similar to those for the mutationally barren branches. And, furthermore, these branches adhered to the normal course of behavior by forming fruit buds for the year to follow. Their close approach to the barren form, when deflorated, suggests that most of the restriction on vegetative growth is imposed by the fruit-development phase of the reproductive process; blooming and fruit-bud formation seem to account for only a relatively minor part of it.

DISCUSSION AND SUMMARY

The results obtained justify certain general conclusions. Barren mutants in the Montmorency cherry, whether as whole trees or as parts of trees, are not, on the whole, lower in photosynthetic activity than normal trees. Their total, seasonal supply of organic materials is not too small for fruit-bud formation, with subsequent blooming and fruit development. This nutrient supply, however, goes directly and completely into vegetative growth, whereas in the normal form it is partly diverted to the requirements of the several phases of sexual reproduction. And this, it may be said, is the basic peculiarity of the genetic condition within the mutant. Affected and fixed in nature by a means known as mutation, it is artificially producible, to some extent, in the normal form by other means, such as defoliation.

The results of the investigation, though far from conclusive, suggest an answer to the questions why, specifically, does the mutant fail to form fruit buds, and why at the critical time for their initiation, does its entire food supply continue to go into vegetative channels? The data seem to show that this is due to a lowered rate of photosynthetic activity which precludes an excess of nutrient materials beyond that required by and taken for the continuation of vegetative growth. In comparisons of the mutant and normal forms the mutant was distinctly below the normal in photosynthetic production just prior to and during the period of detectable fruit-bud differentiation. Moreover, this appears to have been a particular depression in the rate of photosynthesis, for it was clearly below that which obtained after the close of this period and also below that for the entire season. Additional support for this explanation is provided by the analysis of the spurs. Fruit-bud differentiation is not dependent solely on the presence of an excess of organic materials; the location of this excess is also important. Apparently it must occur in the tissues of the spurs as the principal fruit-bud-forming structures of the tree. In the mutant forms these structures were lower than in the normal form in free reducing substances and starch, from early in June—at least a month before observable fruit-bud differentiation—until the end of fruit-bud formation. During this period the mutant's rate of vegetative growth, as shown by table 2, was at its highest, and also at its peak of excess over that of the normal form. Hence, there appears to have been abundant and ample synthesis of organic materials. In the mutant, however, this supply goes promptly into vegetative growth, with relatively slight deposition and accumulation in the spurs. This tendency of the mutant, prohibitive as it is of incipient preparation for fruit-bud initiation, continues throughout the period of fruit-bud differentiation.



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FORMULAS FOR DETERMINING THEORETICAL EFFECTS OF CERTAIN GENETIC FACTORS UPON INHERITANCE OF QUANTITATIVE CHARACTERS, WITH SPECIAL REFERENCE TO A STUDY OF A LYCOPERSICON HYBRID¹

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INTRODUCTION

The universal importance of quantitative characters in plant breeding stresses the desirability of obtaining more information concerning the manner of their inheritance. It is particularly important to know the extent and nature of interactions between the genes differentiating the quantitative characters. Two general methods have been used to determine such interactions. In the first method the segregation of the genes differentiating the quantitative characters is determined by the direct effects that these genes produce. The second method involves the determination of linkages between genes differentiating the quantitative characters and "marker" genes differentiating more simply inherited characters.

Hayes and Harlan (7)³ and Wexelsen (30) used the first or direct method. They determined the major genes involved in the quantitative characters and studied their interactions through certain recognizable genotypes in the segregating populations. Using the same method, Powers (21) separated the 27 genotypes resulting from the segregation of the three factor pairs differentiating habit of growth in crosses between varieties of *Triticum aestivum* L. and determined the nature of the interaction of the genes. A method of setting up the expected limits of fluctuation of any one genotype was given, making it possible to separate the different genotypes and thereby study directly their effects upon habit of growth.

The second method, involving linkage of marker genes with genes differentiating qualitative or simply inherited characters, was first used by Sax (24) and later by Griffiee (4), Sirks (26), Immer (9), Powers (22), Smith (27), and Currence (3). Lindstrom (11, 13, 14, 15, 16) made extensive studies by this method and presented rather conclusive evidence of the existence of major factors differentiating size of fruit in the tomato.

As pointed out by Powers (22), the effects and interactions measured also include any that the marker genes produce as well as the effects of genes linked with them. Studies such as these, in which the marker genes are located on the same chromosome, present sev-

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³ Italic numbers in parentheses refer to Literature Cited, p. 575.

eral problems having a bearing upon the experimental design and upon the analysis and the interpretation of the data. For example, in order to evaluate and interpret accurately the effect of the genes influencing quantitative characters, it is necessary to determine the effect of double crossing over and dominance upon the differences between the means of the genotypes. It also is necessary to determine to what extent the data measure the effect of finite regions of the chromosome and to what extent the data from backcross and from F_2 generations can be used to check each other. This paper presents formulas and their application to a solution of these problems, together with the analysis and the interpretation of data collected on number of locules from the segregates involving *Lycopersicon esculentum* Mill. (Johannisfeuer variety) and *L. pimpinellifolium* Mill. (Red Currant variety).

The formulas apply to situations in which the marker genes and the gene pair affecting the quantitative character are linked in the coupling phase and in which the genes tending to increase the magnitude of the quantitative character are recessive. The symbols used to designate the marker genes are Gg and Oo^{ob} .

FORMULAS FOR DETERMINING THE PROPORTION OF EACH GENOTYPE

Formulas for determining the theoretical proportion of the population that each genotype constitutes are essential to the study. In the development of such formulas those given by Haldane (6) for the proportion of non-cross-overs, single cross-overs, and double cross-overs were used. They are as follows:

No cross-over.....	$(ABC, abc) (1-m) (1-n).$
Cross-over between loci of <i>A</i> and <i>B</i> only.....	$(aBC, Abc) m(1-n).$
Cross-over between loci of <i>B</i> and <i>C</i> only.....	$(ABc, abC) n(1-m).$
Cross-over between loci of <i>A</i> and <i>B</i> and of <i>B</i> and <i>C</i>	$(aBc, AbC) mn.$

In the foregoing formulas, the cross-over value for the section *A* and *B* is m and that for the section *B* and *C* is n . The same notations were used in the development of formulas for determining the theoretical proportion of the population that each genotype constitutes. Bridges and Morgan (1) and Morgan, Bridges, and Sturtevant (20) have shown that the coefficient of coincidence varies between chromosomes and for regions within chromosomes of *Drosophila*. They report that double cross-overs may occur within regions as small as 6 units and again that interference may be complete within distances of 20 units. If double crossing over were influenced only by linkage intensity, theoretically the coefficient of coincidence would be expected to have values ranging from 0 to 1. Weinstein (29) and Bridges and Morgan (1) have shown that values, for the coefficient of coincidence, higher than 1.0 do occur. In the present study the coefficient of coincidence was taken as 1 in developing the formulas for determining the theoretically expected proportion of the population having a particular genotype.

In table 1 are presented the formulas for determining the theoretical proportions of the different genotypes expected among the progeny resulting from crossing the F_1 generation to the parent recessive for the marker genes. The locus of the gene tending to increase the magnitude of the quantitative character is taken as being in that section of the chromosome delimited by the loci of the marker genes.

The last column of table 1 gives the formulas for obtaining the theoretically expected increase in the means of the different genotypes in terms of the quantitative character. Two new notations, v and d , appear in these formulas, v being the amount by which the homozygous condition of the gene pair tending to increase the magnitude of the quantitative character increases the means of the different genotypes, and d being the amount by which the heterozygous condition of the gene pair tending to increase the magnitude of the quantitative character increases the means of the different genotypes. These formulas apply to the data obtained from the progeny of the F_1 backcrossed to the parent recessive for the marker genes. The formulas fulfilling the same purpose for the F_2 generation are given in table 2 and in the tabulation on page 558.

TABLE 1.—Formulas for determining the theoretical proportions of the different genotypes when the locus of the gene tending to increase the quantitative character is between the loci of the two marker genes¹

Genotype	Proportion of genotype		Increase in quantitative character
	Heterozygous	Recessive	
$GgOo^{ab}$ -----	$\frac{(1-m)(1-n)}{2}$	$\frac{mn}{2}$	$\frac{(1-m)(1-n)d+mnv}{(1-m)(1-n)+mn}$
$Ggo^{ab}Oo^{ab}$ -----	$\frac{n(1-m)}{2}$	$\frac{m(1-n)}{2}$	$\frac{n(1-m)d+m(1-n)v}{m+n-2mn}$
$ggOo^{ab}$ -----	$\frac{m(1-n)}{2}$	$\frac{n(1-m)}{2}$	$\frac{m(1-n)d+n(1-m)v}{m+n-2mn}$
$ggo^{ab}Oo^{ab}$ -----	$\frac{mn}{2}$	$\frac{(1-m)(1-n)}{2}$	$\frac{mnd+(1-m)(1-n)v}{(1-m)(1-n)+mn}$

¹ The formulas apply to the progeny obtained from backcrossing the F_1 to the parent recessive for the marker genes.

TABLE 2.—Formulas for determining the theoretical proportions of the different F_2 genotypes when based on the marker genes and given for the homozygous-dominant, heterozygous-dominant, and recessive conditions of a gene pair affecting the quantitative character under investigation, the locus of the gene tending to increase the quantitative character being between the loci of the two marker genes

Genotype	Homozygous dominant	Heterozygous	Homozygous recessive
$GGOO$ ----	$\frac{(1-m)^2(1-n)^2}{4}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{m^2n^2}{4}$
$GGOo^{ab}$ ---	$\frac{n[(1-n)-m(2-m)(1-n)]}{2}$	$\frac{m[(1-m)-2n(1-m)(1-n)]}{2}$	$\frac{m^2n(1-n)}{2}$
$GGo^{ab}Oo^{ab}$ ---	$\frac{n^2(1-m)^2}{4}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{m^2(1-n)^2}{4}$
$GgOO$ ----	$\frac{m[(1-m)-n(1-m)(2-n)]}{2}$	$\frac{n[(1-n)-2m(1-m)(1-n)]}{2}$	$\frac{mn^2(1-m)}{2}$
$GgOo^{ab}$ ---	$\frac{mn(1-m)(1-n)}{2}$	$\frac{1-2m(1-m)-2n(1-n)+4mn(1-m)(1-n)}{2}$	$\frac{mn(1-m)(1-n)}{2}$
$Ggo^{ab}Oo^{ab}$ ---	$\frac{mn^2(1-m)}{2}$	$\frac{n[(1-n)-2m(1-m)(1-n)]}{2}$	$\frac{m[(1-m)-n(1-m)(2-n)]}{2}$
$ggOO$ ----	$\frac{m^2(1-n)^2}{4}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{n^2(1-m)^2}{4}$
$ggOo^{ab}$ ---	$\frac{m^2n(1-n)}{2}$	$\frac{m[(1-m)-2n(1-m)(1-n)]}{2}$	$\frac{n[(1-n)-m(2-m)(1-n)]}{2}$
$ggo^{ab}Oo^{ab}$ ---	$\frac{m^2n^2}{4}$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{(1-m)^2(1-n)^2}{4}$

Formulas for estimating the quantitative increase in the means of the genotypes, due to the effect of 1 gene pair in the F₂ generation

Genotype:	Increase in quantitative character
<i>GGOO</i>	$\frac{2mn(1-m)(1-n)d+m^2n^2v}{(1-m)^2(1-n)^2+2mn(1-m)(1-n)+m^2n^2}$
<i>GGOo^{ob}</i>	$\frac{m[(1-m)-2n(1-m)(1-n)]d+m^2n(1-n)v}{n[(1-n)-m(2-m)(1-n)]+m[(1-m)-2n(1-m)(1-n)]+m^2n(1-n)}$
<i>GGoo^{ob}</i>	$\frac{2mn(1-m)(1-n)d+m^2(1-n)^2v}{n^2(1-m)^2+2mn(1-m)(1-n)+m^2(1-n)^2}$
<i>GgOO</i>	$\frac{n[(1-n)-2m(1-m)(1-n)]d+mn^2(1-m)v}{m[(1-m)-n(1-m)(2-n)]+n[(1-n)-2m(1-m)(1-n)]+mn^2(1-m)}$
<i>GgOo^{ob}</i>	$\frac{[1-2m(1-m)-2n(1-n)+4mn(1-m)(1-n)]d+2mn(1-m)(1-n)v}{1-2m(1-m)-2n(1-n)+8mn(1-m)(1-n)}$
<i>Ggo^{ob}Oo^{ob}</i>	$\frac{n[(1-n)-2m(1-m)(1-n)]d+m[(1-m)-n(1-m)(2-n)]v}{mn^2(1-m)+n[(1-n)-2m(1-m)(1-n)]+m[(1-m)-n(1-m)(2-n)]}$
<i>ggOO</i>	$\frac{2mn(1-m)(1-n)d+n^2(1-m)^2v}{m^2(1-n)^2+2mn(1-m)(1-n)+n^2(1-m)^2}$
<i>ggOo^{ob}</i>	$\frac{m[(1-m)-2n(1-m)(1-n)]d+n[(1-n)-m(2-m)(1-n)]v}{m^2n(1-n)+m[(1-m)-2n(1-m)(1-n)]+n[(1-n)-m(2-m)(1-n)]}$
<i>ggo^{ob}Oo^{ob}</i>	$\frac{2mn(1-m)(1-n)d+(1-m)^2(1-n)^2v}{m^2n^2+2mn(1-m)(1-n)+(1-m)^2(1-n)^2}$

The remaining general condition is that in which the loci of the gene pair affecting the quantitative character studied are outside the *Gg-Oo^{ob}* section of the chromosome. The formulas for determining the theoretical proportions of the different genotypes in which the gene differentiating the quantitative character is located to the left of the *Gg* region are given in table 3, together with the formulas for determining the theoretically expected increase in the means of the different genotypes of the backcross generations. Corresponding formulas for the F₂ generation are given in table 4 and in the tabulation on page 560. The situation in which the gene pair is located to the right of the *Oo^{ob}* region of the chromosome is analogous to that in which the gene pair is located to the left of *Gg*, and therefore it will not be considered in detail.

TABLE 3.—*Formulas for determining the theoretical proportions of the different genotypes when the locus of the gene tending to increase the quantitative character is to the left of the loci of the marker genes*¹

Genotype	Proportion of genotype		Increase in quantitative character
	Heterozygous	Recessive	
<i>GgOo^{ob}</i>	$\frac{(1-m)(1-n)}{2}$	$\frac{m(1-n)}{2}$	$(1-m)d+mv$
<i>Ggo^{ob}Oo^{ob}</i>	$\frac{n(1-m)}{2}$	$\frac{mn}{2}$	
<i>ggOo^{ob}</i>	$\frac{mn}{2}$	$\frac{n(1-m)}{2}$	$md+(1-m)v$
<i>ggo^{ob}Oo^{ob}</i>	$\frac{m(1-n)}{2}$	$\frac{(1-m)(1-n)}{2}$	

¹ The formulas apply to the progeny obtained from backcrossing the F₁ to the parent recessive for the marker genes.

TABLE 4.—*Formulas for determining the theoretical proportions of the different genotypes in the F_2 generation when the locus of the gene tending to increase the quantitative character is to the left of the loci of the two marker genes*

Genotype	Homozygous dominant	Heterozygous	Homozygous recessive
$GGOO$	$\frac{(1-m)^2(1-n)^2}{4}$	$\frac{m(1-m)(1-n)^2}{2}$	$\frac{m^2(1-n)^2}{4}$
GGO_0o^b	$\frac{n(1-m)^2(1-n)}{2}$	$mn(1-m)(1-n)$	$\frac{m^2n(1-n)}{2}$
GGo^bo_0b	$\frac{n^2(1-m)^2}{4}$	$\frac{mn^2(1-m)}{2}$	$\frac{m^2n^2}{4}$
$GgOO$	$\frac{mn(1-m)(1-n)}{2}$	$\frac{n-n^2-2mn(1-m)(1-n)}{2}$	$\frac{mn(1-m)(1-n)}{2}$
GgO_0o^b	$\frac{m(1-m)-2mn(1-m)(1-n)}{2}$	$\frac{1-2m(1-m)-2n(1-n)+4mn(1-m)(1-n)}{2}$	$\frac{n(1-m)-2mn(1-m)(1-n)}{2}$
Ggo^bo_0b	$\frac{mn(1-m)(1-n)}{2}$	$\frac{n-n^2-2mn(1-m)(1-n)}{2}$	$\frac{mn(1-m)(1-n)}{2}$
$ggOO$	$\frac{m^2n^2}{2}$	$\frac{mn^2(1-m)}{2}$	$\frac{n^2(1-m)^2}{2}$
ggO_0o^b	$\frac{m^2n(1-n)}{2}$	$mn(1-m)(1-n)$	$\frac{n(1-m)^2(1-n)}{2}$
ggo^bo_0b	$\frac{m^2(1-n)^2}{2}$	$\frac{m(1-m)(1-n)^2}{2}$	$\frac{(1-m)^2(1-n)^2}{2}$

Formulas for estimating the quantitative increase in the means of the genotypes in the F_2 generation due to the effect of a gene pair to the left of the marker genes

Genotype:		Increase in quantitative character
$GGOO$ -----	}-----	$2m(1-m)d + m^2v$
$GGOo^{ob}$ -----		
$GGo^{ob}o^{ob}$ -----		
$GgOO$ -----	}-----	$[1-2m(1-m)]d + m(1-m)v$
$GgOo^{ob}$ -----		
$Ggo^{ob}o^{ob}$ -----		
$ggOO$ -----	}-----	$\frac{m(1-m)d + (1-m)^2v}{m + (1-m)^2}$
$ggOo^{ob}$ -----		
$ggo^{ob}o^{ob}$ -----		

APPLICATION OF THE FORMULAS

DETERMINING EFFECTS DUE TO REGIONS OF THE CHROMOSOME

With the foregoing formulas available, it is possible to estimate the theoretical amounts by which the means of the different genotypes will be increased as regards the quantitative character under investigation. Also, it is desirable to know what effect varying genetic map distances between the genes affecting both the quantitative and marker characters would have upon these estimates. Therefore, the theoretical estimates of the amounts by which the means of the different genotypes would be increased were calculated for different values of m and n . Further, it is desirable to know what effect different degrees of dominance would have upon these same estimates. The effects of different degrees of dominance can be determined by considering two extreme cases; namely, that in which d equals 0 and in which d equals $v/2$. With these points considered, the theoretical estimates of the amounts by which the means of the different genotypes would be increased by a recessive gene tending to increase the quantitative character and located midway between g and o^{ob} have been calculated and are listed in table 5. The theoretical amounts, or values, are expressed as percentages of v .

First, consider the theoretical proportionate effect of this gene that will be expressed in the means of the different genotypes, when m and n each equals 0.075 and d equals 0 and when the segregates, the means of whose genotypes are being studied, have resulted from backcrossing the F_1 to the recessive parent. From table 5 it can be seen that, theoretically, the mean of the segregates having the genotype $GgOo^{ob}$ has been increased by 0.65 percent of v and that the mean of the segregates having the genotype $ggo^{ob}o^{ob}$ has been increased by 99.35 percent of v . Then the theoretical proportionate effect of the gene tending to increase the magnitude of the quantitative character that can be measured in the progeny obtained by backcrossing to the parent recessive for the marker genes is $ggo^{ob}o^{ob} - GgOo^{ob}$, or 98.7 percent. The loss of 1.3 percent may be attributed to double crossing over. This loss (table 5) increases with an increase in the values of m and n . For the same comparison in which d equals $v/2$, or the heterozygote is intermediate, the theoretical proportionate effect of the gene tending to increase the quantitative character that can be

measured by the comparison $ggo^{ob}o^{ob} - GgOo^{ob}$ is 49.34 percent of v . The difference between this value (49.34) and the same value (98.7) in which d equals 0 measures the effect of dominance, which is 49.36 percent of v . As would be expected, the greater the departure from complete dominance the smaller is the measurable proportion of the effect, in the homozygous condition, of the gene tending to increase the magnitude of the quantitative character.

TABLE 5.—Theoretical increase in the means of the different genotypes, due to a gene located midway between g and o^{ob} and recessive to its allele located between G and O

F₁ BACKCROSSED TO THE RECESSIVE PARENT

Genotype or phenotype	Value of d	Theoretical increase with indicated cross-over values of m and n					
		$m=0.075$ $n=.075$	$m=0.150$ $n=.150$	$m=0.225$ $n=.225$	$m=0.30$ $n=.30$	$m=0.375$ $n=.375$	$m=0.450$ $n=.450$
Genotype:		Percent	Percent	Percent	Percent	Percent	Percent
$GgOo^{ob}$	0	0.65	3.01	7.77	15.52	26.47	40.10
$Ggo^{ob}o^{ob}$		50.00	50.00	50.00	50.00	50.00	50.00
$ggOo^{ob}$		50.00	50.00	50.00	50.00	50.00	50.00
$ggo^{ob}o^{ob}$		99.35	96.99	92.23	84.48	73.53	59.90
$GgOo^{ob}$	$v/2$	50.33	51.50	53.88	57.76	63.24	70.05
$Ggo^{ob}o^{ob}$		75.00	75.00	75.00	75.00	75.00	75.00
$ggOo^{ob}$		75.00	75.00	75.00	75.00	75.00	75.00
$ggo^{ob}o^{ob}$		99.67	98.50	96.12	92.24	86.76	79.95

F₂ GENERATION¹

Phenotype: ¹							
$GgOo^{ob}$	0	0.76	3.01	6.58	11.13	16.27	21.57
$Ggo^{ob}o^{ob}$		47.83	45.06	41.65	37.66	33.16	28.32
$ggOo^{ob}$		47.83	45.06	41.65	37.66	33.16	28.32
$ggo^{ob}o^{ob}$		98.70	94.05	85.06	71.37	54.06	35.88
$GgOo^{ob}$	$v/2$	32.35	32.81	34.70	37.84	41.96	46.68
$Ggo^{ob}o^{ob}$		72.83	70.06	66.65	62.66	58.16	53.32
$ggOo^{ob}$		72.83	70.06	66.65	62.66	58.16	53.32
$ggo^{ob}o^{ob}$		99.35	96.98	92.23	84.48	73.53	59.90

¹ The homozygous dominants are included with the heterozygous dominants.

Turning to the F₂ generation in which the homozygous-dominant and heterozygous-dominant segregates have not been separated and in which d equals 0, the effects of double crossing over are not great when m and n have small values (0.075 or 0.150), and therefore a fairly accurate measure of the effect of the gene tending to increase the quantitative character is obtained by the comparison $ggo^{ob}o^{ob} - GgOo^{ob}$ (table 5). In drawing other conclusions from the F₂ data presented in table 5, it must be kept in mind that the homozygous-dominant and heterozygous-dominant segregates were not separated.

The theoretical estimates of the amount by which the means of the different genotypes will be increased in respect to the quantitative character under investigation for the F₂ generation in which the homozygous dominants and heterozygous dominants have been separated are given in table 6. More than 90 percent of the theoretical expected effect of a gene tending to increase the magnitude of the quantitative character is measured by the comparison $ggo^{ob}o^{ob} - GGOO$ when d equals 0 or $v/2$ and when the values of m and n do not exceed 0.150.

TABLE 6.—Theoretical effects in percent upon the means of all F_2 genotypes of a gene located midway between g and o^{ob} and tending to increase the quantitative character but recessive to its allele located between G and O

Value of d and genotype	Theoretical effects with indicated cross-over values of m and n					
	$m=0.075$ $n=.075$	$m=0.150$ $n=.150$	$m=0.225$ $n=.225$	$m=0.30$ $n=.30$	$m=0.375$ $n=.375$	$m=0.45$ $n=.45$
$d=0$:	Percent	Percent	Percent	Percent	Percent	Percent
$GGOO$	0.01	0.09	0.60	2.41	7.01	18.08
$GGOo^{ob}$	0.33	1.51	3.89	7.76	13.24	20.05
$GGo^{ob}o^{ob}$	25.00	25.00	25.00	25.00	25.00	25.00
$GgOO$	0.33	1.51	3.89	7.76	13.24	20.05
$GgOo^{ob}$	1.27	5.24	11.14	17.20	21.89	24.50
$Ggo^{ob}o^{ob}$	49.67	48.49	46.11	42.24	36.76	29.95
$ggOO$	25.00	25.00	25.00	25.00	25.00	25.00
$ggOo^{ob}$	49.67	48.49	46.11	42.24	36.76	29.95
$ggo^{ob}o^{ob}$	98.70	94.05	85.06	71.37	54.06	35.88
$d=v/2$:						
$GGOO$65	3.02	7.77	15.52	26.47	40.10
$GGOo^{ob}$	25.33	26.51	28.89	32.76	38.24	45.05
$GGo^{ob}o^{ob}$	50.00	50.00	50.00	50.00	50.00	50.00
$GgOO$	25.33	26.51	28.89	32.76	38.24	45.05
$GgOo^{ob}$	50.00	50.00	50.00	50.00	50.00	50.00
$Ggo^{ob}o^{ob}$	74.67	73.49	71.11	67.24	61.76	54.95
$ggOO$	50.00	50.00	50.00	50.00	50.00	50.00
$ggOo^{ob}$	74.67	73.49	71.11	67.24	61.76	54.95
$ggo^{ob}o^{ob}$	99.35	96.98	92.23	84.48	73.53	59.90

So far the discussion has been limited to calculations in which the position of the gene tending to increase the magnitude of the quantitative character was taken as midway between the loci of the two gene pairs differentiating the qualitative character. Tables 7 and 8 give the theoretically expected amounts by which the means of the different genotypes would be increased when the values of m and n are not equal. In the examples chosen the gene pair tending to increase the magnitude of the quantitative character is taken (in genetic-map units) as being located closer to g than to o^{ob} . An inspection of tables 7 and 8 shows that the conclusions concerning the proportionate amounts of the effect of the gene pair (in the homozygous condition) tending to increase the magnitude of the quantitative character that is measured by the comparisons $ggo^{ob}o^{ob}-GgOo^{ob}$ and $ggo^{ob}o^{ob}-GGOO$ are the same as those drawn for the calculations (tables 5 and 6) made on the basis that the gene affecting the quantitative character is located midway between g and o^{ob} . When neither m nor n exceeded a value of 0.150 and d equaled 0, the comparison $ggo^{ob}o^{ob}-GgOo^{ob}$ for the backcross and the comparison $ggo^{ob}o^{ob}-GGOO$ for the F_2 generation measured more than 95 percent of the effect of the gene in the homozygous condition. This same statement is true for that part of the F_2 generation in which the homozygous-dominant and heterozygous-dominant segregates were separated and d equaled $v/2$ (table 8).

In the development of the formulas the coefficient of coincidence was taken as 1, which is seldom, if ever, the case when the values of m and n are small. Therefore, in actual experiments the effect of double crossing over upon the proportionate amount by which the means of the different genotypes would be increased probably is less than is shown by the data of tables 5 to 8, inclusive.

TABLE 7.—Theoretical effects in percent upon the means of the different genotypes of a gene located closer to *g* than to *o^{ob}* in the section between *g* and *o^{ob}* and tending to increase a quantitative character but recessive to its allele located between *G* and *O*F₁ BACKCROSSED TO THE RECESSIVE PARENT

Genotype	Theoretical effects, with indicated cross-over values of <i>m</i> and <i>n</i> , where—					
	<i>d</i> =0			<i>d</i> = <i>v</i> /2		
	<i>m</i> =0.075 <i>n</i> = .150	<i>m</i> =0.15 <i>n</i> = .30	<i>m</i> =0.30 <i>n</i> = .45	<i>m</i> =0.075 <i>n</i> = .150	<i>m</i> =0.15 <i>n</i> = .30	<i>m</i> =0.30 <i>n</i> = .45
	Percent	Percent	Percent	Percent	Percent	Percent
<i>GgOo^{ob}</i>	1.41	7.03	25.96	50.71	53.52	62.98
<i>Ggo^{ob}Oo^{ob}</i>	31.48	29.17	34.37	65.74	64.58	67.19
<i>ggOo^{ob}</i>	68.52	70.83	65.63	84.26	85.42	82.81
<i>ggo^{ob}Oo^{ob}</i>	98.59	92.97	74.03	99.29	96.48	87.02

F₂ GENERATION¹

<i>GgOo^{ob}</i>	1.52	5.87	15.67	32.32	34.48	41.85
<i>Ggo^{ob}Oo^{ob}</i>	28.66	23.03	21.14	61.26	54.06	47.94
<i>ggOo^{ob}</i>	65.23	62.41	46.84	81.86	79.47	68.50
<i>ggo^{ob}Oo^{ob}</i>	97.20	86.43	54.82	98.59	92.97	74.04

¹ The homozygous-dominants are included with the heterozygous dominants.TABLE 8.—Theoretical effects upon the means of all F₂ genotypes of a gene located closer to *g* than to *o^{ob}* in the section between *g* and *o^{ob}* and tending to increase a quantitative character but recessive to its allele located between *G* and *O*

Genotype	Theoretical effects, with indicated cross-over values of <i>m</i> and <i>n</i> , where—					
	<i>d</i> =0			<i>d</i> = <i>v</i> /2		
	<i>m</i> =0.075 <i>n</i> = .150	<i>m</i> =0.15 <i>n</i> = .30	<i>m</i> =0.30 <i>n</i> = .45	<i>m</i> =0.075 <i>n</i> = .150	<i>m</i> =0.15 <i>n</i> = .30	<i>m</i> =0.30 <i>n</i> = .45
	Percent	Percent	Percent	Percent	Percent	Percent
<i>GGOO</i>	0.02	0.49	6.74	1.41	7.03	25.96
<i>GGOo^{ob}</i>44	2.05	8.93	16.45	18.10	30.17
<i>GGo^{ob}Oo^{ob}</i>	9.91	8.51	11.82	31.48	29.17	34.37
<i>GgOO</i>97	4.98	17.04	34.96	38.93	45.79
<i>GgOo^{ob}</i>	2.61	9.93	20.76	50.00	50.00	50.00
<i>Ggo^{ob}Oo^{ob}</i>	31.04	27.12	25.45	65.04	61.07	54.21
<i>ggOO</i>	46.95	50.17	43.07	68.52	70.83	65.63
<i>ggOo^{ob}</i>	67.55	65.85	48.59	83.55	81.90	69.83
<i>ggo^{ob}Oo^{ob}</i>	97.20	86.43	54.82	98.59	92.97	74.04

DETERMINING INTERACTIONS AND THEIR NATURE

In a study of the interactions of the genes affecting the quantitative characters and located at different loci of the same chromosome, it is necessary to know whether the effects of double crossing over and dominance are confounded with the interactions, and if so in what generations. As regards the interactions there are three possible situations. (1) There may not be any interaction between the genes differentiating the quantitative character. In such a case, the effects of the different gene pairs tending to increase the magnitude of the quantitative character will be strictly additive when combined. (2) Upon combination, the nature of the interaction of the genes may be such "that the effect of each factor on the genotype is dependent

upon all the other factors present, the visible effect of a certain factor being smaller the greater the number of factors acting in the same direction" (23). (3) The nature of the interaction of the genes differentiating the quantitative character may be such that the genes tending to increase the magnitude of the quantitative character may give greater differences over their alleles in combination with genes also tending to increase the magnitude of the quantitative character than when in combination with the alleles of these genes (22). In testing for and studying the nature of the interaction of factors the following comparison is used: $(Ggo^{ob}o^{ob} - GgOo^{ob}) - (ggo^{ob}o^{ob} - ggOo^{ob})$.

Consider first the F_1 generation backcrossed to the recessive parent. In all cases, barring possible differences due to dropping of figures to the right of the decimal point, the comparison $(Ggo^{ob}o^{ob} - GgOo^{ob}) - (ggo^{ob}o^{ob} - ggOo^{ob})$ equals 0. That such must be the case can be proved readily by substituting the formulas given in table 1 for the corresponding genotypes and solving the equation. Then it is apparent that neither the effects of double crossing over nor of dominance are confounded with the interactions.

Turning to the F_2 generation, it can be seen that when m and n each equals 0.075 and d equals 0 the comparison $(ggo^{ob}o^{ob} - ggOO) - (GGO^{ob}o^{ob} - GG00)$ equals 48.71 percent and not 0 (table 6). Obviously, without the aid of table 6 the discrepancy between the differences might have been interpreted as an interaction of genes differentiating the quantitative character. The discrepancy of 48.71 percent is not directly due to the effect of double crossing over nor the degree of dominance, but is attributable to recombination of gametes containing certain single cross-overs. The discrepancies are small and consequently not so serious when the comparisons involve only the heterozygous dominants and the homozygous recessives, for example the comparison $(ggo^{ob}o^{ob} - ggOo^{ob}) - (Ggo^{ob}o^{ob} - GgOo^{ob})$, of table 6, in which d equals 0 and m and n each equals 0.150. The same comparisons (tables 6 and 8) in which d equals $v/2$ show that neither the effects of double crossing over nor the effects of dominance are confounded with possible effects due to interactions, but that the effects due to recombination of gametes containing certain single cross-overs are confounded with the effects due to possible interactions of genes differentiating quantitative characters. Therefore, in testing for interactions and in studying the nature of the interactions of genes differentiating quantitative characters by means of their linkage relationship with marker genes, in which all the genes involved have their loci on the same or homologous chromosomes, allowances must be made, in an interpretation of the results, for the effects due to recombination of gametes containing certain single cross-overs.

With the foregoing information available, it is possible to determine to what extent the effect of finite regions of the chromosomes is measured by following the segregation of the genes differentiating the quantitative characters by means of their linkage relations with the marker genes. To do this it is necessary to know what effect genes located to the left of the Gg region of the chromosome would have upon the means of the OO , Oo^{ob} , and $o^{ob}o^{ob}$ segregates and what effect genes located to the right of the Oo^{ob} region of the chromosome would have upon the means of the GG , Gg , and gg segregates. Since the two

situations are analogous, only one need be considered in detail. For example, take the case in which the additional genes affecting the quantitative character are located to the left of Gg . The formulas for determining the theoretical proportions of the population and the theoretically expected amounts by which the means of the different genotypes would be increased are given in tables 3 and 4 and in the tabulation on page 560. Table 3 gives the formulas applicable to the F_1 generation backcrossed to the parent recessive for the marker genes. From the column "Increase in quantitative character" it can be seen that the formulas are the same for the genotypes $GgOo^{ob}$ and $Ggo^{ob}o^{ob}$ and for the genotypes $ggOo^{ob}$ and $ggo^{ob}o^{ob}$. In the tabulation (p. 560) giving the formulas applicable to the F_2 generation, the formulas are shown to be the same for the genotypes $GGOO$, $GGOo^{ob}$, and $GGo^{ob}o^{ob}$; for the genotypes $GgOO$, $GgOo^{ob}$, and $Ggo^{ob}o^{ob}$; and for the genotypes $ggOO$, $ggOo^{ob}$, and $ggo^{ob}o^{ob}$. Such being the case, it is evident that genes located to the left of the Gg region of the chromosome have no effect upon the means of OO , Oo^{ob} , or $o^{ob}o^{ob}$ segregates when the comparisons are made within a particular genotype resulting from a segregation of the Gg alleles. Likewise, for similar comparisons the means of the GG , Gg , and gg genotypes are not affected by genes having their loci to the right of Oo^{ob} .

The foregoing discussion has already largely brought out to what extent the data from the F_1 backcrossed to the parent recessive for the marker genes and the data from the F_2 generation can be used as checks against each other. The percentages tabulated in tables 5, 6, 7, and 8 show that within limits the data from one can be used in checking the conclusions drawn from the data of the other, if the formulas listed in tables 1 to 4 and in the tabulations on pages 558 and 560 are applied and the results used as an aid in an interpretation of the data.

A THREE-POINT EXPERIMENTAL DESIGN

The information derived from the foregoing tables aids materially in designing an experiment to study the nature of the interaction of genes located in the same chromosome and differentiating quantitative characters. The study of the nature of the interaction of the quantitative genes can be made by means of their linkage with marker genes. For example, consider three gene pairs serving as markers and designated as Gg , Oo^{ob} , and Hh . In addition, suppose the linkage relationship of these three gene pairs to be as follows:

$$\frac{G \ O \ H}{g \ o^{ob} \ h}$$

Further, let it be stipulated that double cross-overs do not occur within the section delimited by any two adjacent nonallelic genes. The different regions of the chromosome shall be designated as Gg , Oo^{ob} , and Hh . The design is that of a three-point experiment in which the linkage intensity is sufficient to prevent double crossing over within sections of the chromosomes delimited by adjacent nonallelic genes.

The efficacy of the three-point experiment, as a design for determining the effect of different regions of the chromosome upon the quantitative character under investigation and for determining the

nature of the interaction of the genes affecting this character by means of their linkage with marker genes, needs to be determined. Also, the relationship between the effect of the different regions (*Gg*, *Oo^{ob}*, and *Hh*) is important. For example, consider the population derived from the backcross of the F_1 generation to the parent recessive for all three of the gene pairs differentiating the simply inherited characters. Eight different genotypes should appear among the progeny of such a backcross, namely:

GgOo^{ob}Hh
GgOo^{ob}hh
Ggo^{ob}Oo^{ob}Hh
ggOo^{ob}Hh

Ggo^{ob}Oo^{ob}hh
ggOo^{ob}hh
ggo^{ob}Oo^{ob}Hh
ggo^{ob}Oo^{ob}hh

The problem involved is how the comparison between the different genotypes should be interpreted. For example, the difference *GgOo^{ob}Hh*—*Ggo^{ob}Oo^{ob}Hh* could be due to a gene affecting the quantitative character under investigation and located in either the section delimited by *Gg* and *Oo^{ob}* or the section delimited by *Oo^{ob}* and *Hh*. Also, the effect could be due to the loci designated as *O* and *o^{ob}*. If the gene or genes having an effect upon the quantitative character are located between *Gg* and *Oo^{ob}*, the *Gg* region of the chromosome, as has been previously shown, would also have an effect upon the quantitative character due to the same gene or genes. For example, suppose that the difference between the gene having an effect upon the quantitative character in the heterozygous condition had a value of 0, as compared with its allele in the homozygous recessive condition having a value of 4. Under such specifications, the difference between *Ggo^{ob}Oo^{ob}Hh* and *GgOo^{ob}Hh* would be 2 and the difference between *ggOo^{ob}Hh* and *GgOo^{ob}Hh* would be 2 also. Their sum would give the total effect of the gene considered in the homozygous recessive condition. As has been shown in a previous section of this paper, the gene pair whose effect is being studied need not be located midway between the gene pairs *Gg* and *Oo^{ob}* for the differences to add up to the total effect of the gene in the homozygous condition. If the gene occupied any locus in the *Gg*—*Oo^{ob}* section of the chromosome, the results would be the same, under the stipulations, namely, that double cross-overs do not occur in the section between *Gg* and *Oo^{ob}*. It should not be concluded that the comparison (*Ggo^{ob}Oo^{ob}Hh*—*GgOo^{ob}Hh*) + (*ggOo^{ob}Hh*—*GgOo^{ob}Hh*) measures only the effects of genes located between the gene pairs *Gg* and *Oo^{ob}*, as any genes to the left of *Gg* and to the right of *Oo^{ob}* and to the left of *Hh* would have an effect. However, it is apparent that the comparison (*Ggo^{ob}Oo^{ob}Hh*—*GgOo^{ob}Hh*) + (*ggOo^{ob}Hh*—*GgOo^{ob}Hh*) must include the total effect of the *Oo^{ob}* region, and it is equally apparent that the effect of two regions, whose effects have been obtained in such a manner, can be summed to give the total effect of the two regions, provided an interaction of the genes differentiating the quantitative characters does not exist.

This leads to the problem of testing for and evaluating the interactions. It can be seen from the foregoing examples that the comparison (*Ggo^{ob}Oo^{ob}Hh*—*GgOo^{ob}Hh*)—(*ggo^{ob}Oo^{ob}Hh*—*ggOo^{ob}Hh*) does not necessarily represent a test for an interaction, because, if only one gene pair differentiating the quantitative character being considered were involved, the value obtained would be 0 (see table 5, F_1 backcrossed to the recessive parent), which is the same value as would be

obtained if more than one gene pair were affecting the quantitative character and no interaction of the genes existed. However, the comparison ($ggOo^{ob}Hh - GgOo^{ob}Hh$) — ($ggOo^{ob}hh - GgOo^{ob}hh$) does constitute a test for interactions and does offer a means of testing the nature of these interactions, provided the Hh region of the chromosome has been shown to have an effect, because the differences between gg and Gg in such a comparison are not affected by genes located to the right of Oo^{ob} other than through possible interactions. Therefore, it is obvious that the three-point experiment as outlined is adapted to studying the nature of the interaction of genes differentiating quantitative characters.

In conclusion it should be pointed out that, even though more than three gene pairs differentiating more than three marker characters are involved in the study, the three-point experiment is very useful, since larger numbers of individuals will be available for estimating the value of each genotype than if an attempt were made to study all regions simultaneously. However, more information concerning the interactions can be obtained, if adequate numbers are available to estimate the means for each genotype, by considering all regions of the chromosomes in one extensive analysis. But such a utopian situation as adequate numbers for all genotypes possible from a segregation of more than three linked gene pairs is seldom realized in working with plant material.

NUMBER OF LOCULES IN TOMATOES

The purpose of the preceding formulas and tables is to aid in the interpretation of data on the inheritance of quantitative characters. They apply to certain cases in which regions of homologous chromosomes are being studied. A study on the inheritance of number of locules in tomatoes offered an opportunity for the practical application of the information derived from the foregoing study. The results obtained are reported herewith.

The study on the inheritance of number of locules in tomatoes was made from a cross involving *Lycopersicon esculentum* var. *Johannisfeuer* (the many-loculed parent) and *L. pimpinellifolium* var. *Red Currant* (the few-loculed parent). Data were taken from the F_2 generation, the F_1 generation backcrossed to both parents, the F_1 generation, and both parents. Owing to the limited number of F_1 plants available, they were grown in a separate randomized experiment with both parents. In the F_2 generation, both backcrosses and the parents were grown in a randomized block experiment, consisting of rows having 24 plants each, with $3\frac{1}{2}$ feet between plants within the row and the same distance between rows. The rows per block were distributed as follows: The F_2 generation, 16 rows; the backcross to *Red Currant*, 4 rows; the backcross to *Johannisfeuer*, 12 rows; *Johannisfeuer*, 2 rows; and *Red Currant*, 3 rows. The rows were randomized within blocks by the use of Tippet's (28) tables, and a total of 9 blocks was used in the analysis. The method of analysis was that given by Powers (22), in which the t test was used to determine statistically significant differences.

Of importance in an interpretation of the data are (1) dominance of the genes affecting the quantitative character as well as those used as marker genes, (2) intensity of the linkage relationship of the genes

used as markers, and (3) number of factor pairs involved in differentiating the characters used as markers.

As previously stated, the quantitative character under consideration was number of locules. Depth of grooves and shape of fruit were the two characters whose segregation in the two different generations made it possible to determine the major gene pairs involved in their differentiation and the linkage relationships. The characters number of locules and depth of grooves are shown in figure 1. It will be remembered that the F_1 generation plants were grown in comparison with plants of both parents and are not directly comparable with the parents grown for comparison with the F_2 and backcross generation plants. The data given in explanation of figure 1 are comparable. The Johannisfeuer parent averaged 9.60 locules per fruit, the F_1 generation 2.40 locules per fruit, and the Red Currant parent 2.00 locules

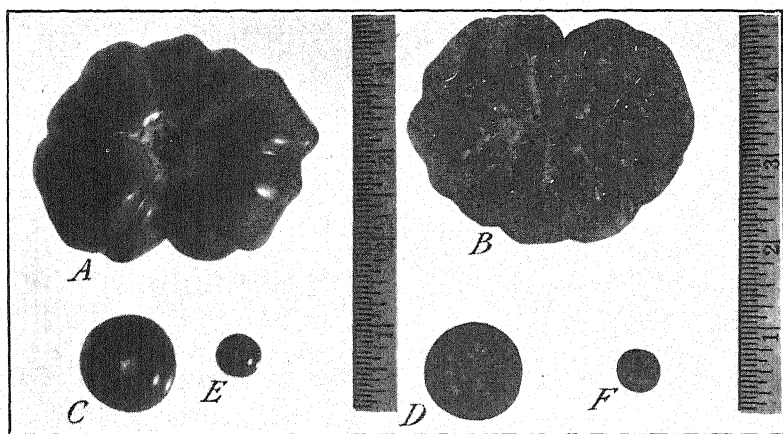


FIGURE 1.—Tomato hybrid and parents: A, B, Johannisfeuer parent; C, D, F_1 generation; E, F, Red Currant parent. Mean number of locules: Johannisfeuer, 9.60; F_1 generation, 2.40; Red Currant, 2.00. Left (A, C, E) shows smooth type dominant.

per fruit. The differences between the mean number of locules for Johannisfeuer and the mean number of locules for either the F_1 generation or the Red Currant parent are statistically significant; but the difference between the mean number of locules for the F_1 generation and the mean number of locules for the Red Currant parent does not reach statistical significance if odds of 19:1 against the deviations noted as due to the probable errors of random sampling are accepted as a criterion. It is apparent that the character few locules per fruit is completely dominant or nearly so. The photographs of the three fruits to the left in figure 1 (A, C, E) show that smooth type of fruit is almost completely dominant to fruit with deep grooves.

Next, consider shape of fruit (fig. 2). To facilitate the comparison of shapes, the photographs of the three fruits have been brought to approximately the same diameter. It can be seen that the fruit of the Red Currant parent is essentially round, that of the F_1 generation somewhat oblate, and that of the Johannisfeuer parent decidedly oblate. It was possible to separate the homozygous dominants from

the heterozygous dominants in the generations segregating for fruit shape; but the separation is not shown in the F_2 generation because of the inadequate number of some genotypes.

The segregation for depth of grooves and shape of fruit will be considered next. The ratio of smooth-fruited plants to those having deep grooves was 2,508:871 in the F_2 generation and 1,272:1,229 among the progeny of the F_1 backcrossed to Johannisfeuer; all of the plants from the F_1 generation backcrossed to Red Currant had smooth fruits. As to shape of fruit, the ratio of round fruit to somewhat oblate fruit to decidedly oblate fruit was 851:1,651:877 for the F_2

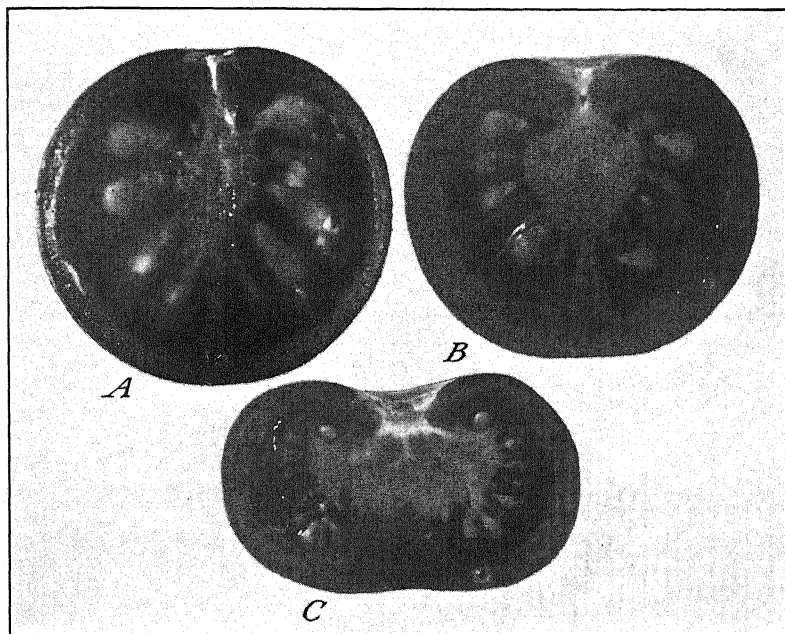


FIGURE 2.—Tomato hybrid and parents. A, Red Currant parent; shape, round. B, F_1 generation; shape, somewhat oblate. C, Johannisfeuer parent; shape, decidedly oblate.

generation; the ratio of somewhat oblate to decidedly oblate was 1,249:1,252 for the progeny of the F_1 backcrossed to Johannisfeuer; and the ratio of round to somewhat oblate was 410:438 for the F_1 backcrossed to Red Currant. It is apparent that depth of grooves and shape of fruit are each differentiated by one major factor pair. Lindstrom (12, 13) believes that a series of multiple alleles governs fruit shape in tomatoes. Since he found that round and oval shape of fruit segregated as expected on the basis of a one-factor pair, and since the segregation for round and oblate shape of fruit reported herein is that expected on the basis of a one-factor pair, the present study offers further support for the deductions made by Lindstrom.

Turning to the linkage intensities, the major factor pair differentiating depth of grooves was found to be linked with the major factor pair differentiating shape of fruit, with 18 ± 0.76 percent of crossing over in the F_2 generation and 15 ± 0.71 percent of crossing over among

the progeny obtained from crossing the F_1 generation back to Johannisfeuer. Immer's (10) formulas were used in calculating the cross-over values and standard errors. The linkage was in the coupling phase. The symbols used to designate the genes involved were as follows:

G —symbol used for the gene differentiating smooth from deeply grooved type of fruit.

g —symbol used for the allele of G .

O —symbol used for the gene differentiating round from oblate type of fruit.

o^{ob} —symbol used for the allele of O .

With the foregoing data on the inheritance of the different characters, it is possible to determine the effect of the Gg and Oo^{ob} regions of the chromosome upon number of locules and to ascertain whether the genes located in the two regions interact and, if so, the nature of the interaction. The linkage intensities of 18 percent for the F_2 generation and 15 percent for the backcross generation, the almost complete dominance exhibited by the F_1 generation as compared to the parents, and the almost complete lack of difference between the means of the $Ggo^{ob}o^{ob}$ and $ggOo^{ob}$ genotypes make it appear that those data of table 5, column 3, in which d equals 0 furnish a satisfactory estimate of the effect that the maximum expected amount of double crossing over and the degree of dominance would have upon the means of the different genotypes, and therefore they should be of value as a guide in interpreting the data given in tables 9 and 10. To facilitate comparisons, the values referred to (table 5) have been copied in column 3 of table 9.

TABLE 9.—Mean number of locules for the different generations, genotypes, and parents of the cross *Lycopersicon esculentum* var. *Johannisfeuer* \times *L. pimpinellifolium* var. *Red Currant*

Generation, genotype, and parent	Obtained mean number	Theoretical percentage of v^1	Generation, genotype, and parent	Obtained mean number	Theoretical percentage of v^1
F_2 generation: ²			F_1 backcrossed to Red Currant:		
$GgOo^{ob}$	2.52	0.76	Og^{ob}	2.21	-----
$Ggo^{ob}o^{ob}$	3.51	47.83	$o^{ob}g^{ob}$	2.36	-----
$ggOo^{ob}$	3.52	47.83	Parents:		
$ggo^{ob}o^{ob}$	5.22	98.70	Red Currant.....	2.04	-----
F_1 backcrossed to Johannisfeuer:			Johannisfeuer.....	10.27	-----
$GgOo^{ob}$	3.61	.65			
$Ggo^{ob}o^{ob}$	5.04	50.00			
$ggOo^{ob}$	5.03	50.00			
$ggo^{ob}o^{ob}$	7.48	99.35			

¹ See table 5, column 3, in which $d=0$.

² The homozygous dominants and heterozygous dominants are not separated.

Column 3 of table 9 shows that, on the basis of only one gene pair differentiating plants with few-loculed fruits from plants with many-loculed fruits, the means of the $Ggo^{ob}o^{ob}$ and $ggOo^{ob}$ genotypes should be identical. From column 2 of table 9 it can be seen that the means for these two genotypes in both the F_2 and backcross generations are almost identical. It should be pointed out that the differences between means of these two genotypes in both the F_2 and backcross generations were smaller than would be expected on the basis of the t test. As the variance of error was estimated for within genotypes

and blocks, ignoring replication, the discrepancy may be due to the fact that differences between means of replications were not removed from an estimate of the variance. The reason for not removing the

TABLE 10.—Main effects and interactions of different regions of the chromosome and of different generations upon number of locules in the cross *Johannisfeuer* × *Red Currant*¹

Item	Differences for within generations and genotypes			
	F ₂		B ₁ ²	
Main effects:				
Regions:				
<i>gg—Gg</i>	<i>Oo^{ob}</i> 1.00	<i>o^{ob}go^{ob}</i> 1.71	<i>Oo^{ob}</i> 1.42	<i>o^{ob}go^{ob}</i> 2.44
<i>o^{ob}go^{ob}—Oo^{ob}</i>	<i>Gg</i> .99	<i>gg</i> 1.70	<i>Gg</i> 1.43	<i>gg</i> 2.45
Generations:	Genotype			
² B ₁ —F ₂	<i>GgOo^{ob}</i> 1.09	<i>Ggo^{ob}go^{ob}</i> 1.53	<i>ggOo^{ob}</i> 1.51	<i>ggo^{ob}go^{ob}</i> 2.26
Interactions:	First order			
<i>Gg×Oo^{ob}</i>	F ₂ .71	B ₁ ² 1.02		
<i>Gg×generations</i>	<i>Oo^{ob}</i> .42	<i>o^{ob}go^{ob}</i> .73		
<i>Oo^{ob}×generations</i>	<i>Gg</i> 3.44	<i>gg</i> 3.75		
	Second order			
<i>Gg×Oo^{ob}×generations</i>	4.31			

¹ The *t* test gives a value of $P < 0.01$ for all values not otherwise designated.

² B₁ designates the progeny from the F₁ backcrossed to the recessive parent, *Johannisfeuer*.

³ $P > 0.01$ but < 0.02 .

⁴ $P > 0.05$.

variance due to differences between means of replications was that the number of plants of some genotypes was small for the replications. The fact that the means of the *Ggo^{ob}o^{ob}* and *ggOo^{ob}* genotypes are almost identical would indicate that only one gene pair located midway between *Gg* and *Oo^{ob}* affecting number of locules is segregating in this cross. Such being the case, certain conclusions would necessarily follow. The difference *gg—Gg* should equal the difference *o^{ob}go^{ob}—Oo^{ob}*; the differences should be approximately the same for all genotypes and generations, or, in other words, there would be no interactions and the values listed under interactions in table 10 should not be statistically significant; there should not be any statistically significant differences between the means of the F₂ and backcross generations as regards the *ggo^{ob}o^{ob}* genotype; and finally the mean of the *GgOo^{ob}* genotype should be a very close approximation of the mean of the *Red Currant* parent and the mean of the *ggo^{ob}o^{ob}* genotype should approximate very closely that of the *Johannisfeuer* parent.

To facilitate a study of whether the obtained data meet the foregoing specifications, the differences were tabulated in table 10. The

differences between alleles and the differences between generations are statistically significant. The latter fact is contrary to the specification that there should be no differences between generations as regards the $ggo^{ob}o^{ob}$ genotype. Of the differences listed under interactions, all of the first-order interactions are statistically significant, whereas the triple interaction is not. This is contrary to the specification that none of the interactions of table 10 should be statistically significant. Finally, refined statistical analysis is not necessary to show that the two means for the $GgOo^{ob}$ genotype are significantly larger than the mean of the Red Currant parent and that the two means for the $ggo^{ob}o^{ob}$ genotype are smaller than the mean of the Johannisfeuer parent. Clearly, the segregation of a single factor pair will not explain the data. Some other interpretation must be sought.

From table 10, it can be seen that the differences between the means given for the generation obtained by backcrossing the F_1 to the Johannisfeuer parent and the means given for the F_2 generation are nearly equal to the differences for the comparisons $gg-Gg$ and $o^{ob}o^{ob}-Oo^{ob}$. This shows that at least one other gene pair, not closely associated with the Gg or Oo^{ob} regions of the chromosome, has an effect upon number of locules and is segregating in this cross. Likewise, the fact that the interaction $Gg \times Oo^{ob}$ is statistically significant shows that more than one locus of the chromosome carrying the alleles Gg and Oo^{ob} is affecting number of locules. From these results it is very evident that at least three gene pairs affecting number of locules are segregating in this cross.

Such being the case further consideration should be given to the fact that the differences ($gg-Gg$) and ($o^{ob}o^{ob}-Oo^{ob}$) were so nearly equal, indicating that only one major factor pair affecting number of locules was segregating in the cross studied. Yeager (31) has shown that in the material with which he worked a major factor pair affecting number of locules was linked with the gene pair differentiating shape of fruit. However, his many-loculed parents had from 4 to 5 locules, whereas in the present study the many-loculed parent averaged 10.27 locules per fruit. The few-loculed parents had from 2 to 3 locules, as did the few-loculed parents in the present study. From these considerations, it is apparent that Johannisfeuer, the many-loculed parent involved in the cross reported herein, probably is not of the same genetic constitution as the many-loculed parents studied by Yeager. However, since Yeager has shown that a major factor pair affecting number of locules is linked with a gene pair differentiating shape of fruit, and since the writer found a major effect linked with shape of fruit, it seems probable that Johannisfeuer carries the same gene pair affecting number of locules that was reported for No. 15 and Bison by Yeager (31). Furthermore, the fact that the differences ($gg-Gg$) and ($o^{ob}o^{ob}-Oo^{ob}$) are so nearly equal is an indication that this gene pair, *Lclc* (Yeager, 31) is located midway between the Gg loci and the Oo^{ob} loci of the chromosome. Then, the data (as regards chromosome 1 of the tomato) obtained in the cross Johannisfeuer \times Red Currant can be explained by the *Lclc* gene pair plus other gene pairs located in the $Gg-Oo^{ob}$ section of the chromosome or located to the right or left of this section or both, and balanced numerically or qualitatively or both, so as to give the results noted.

Since it has been shown that at least three gene pairs affecting number of locules are segregating in the cross *Johannisfeuer* \times Red Currant, the nature of the interactions of the genes associated with the *Gg* and *Oo^{ob}* regions of the chromosome can be determined. However, before making an interpretation of the data it is desirable to have some findings of previous investigators in mind. Mangelsdorf and Fraps (19) found that the vitamin A units per gram increase approximately 2.25 for each additional *Y* gene. Groth (5) as early as 1915 believed that a geometric progression was important in the inheritance of quantitative characters. Lindstrom (17), from studies to determine the nature of the interaction of the genes affecting size of fruit involving crosses between species of *Lycopersicum*, realized clearly the importance of not confounding the interaction between pairs of alleles with dominance and the probability that the nature of the interactions of the genes may be such as to result in a geometric progression. His conclusions (17, p. 10) follow:

In concluding, the writer does not mean to imply that geometric action of genes for quantitative characters is not an important aspect. On the contrary, such action is to be expected. But he does wish to point out that genes for quantitative characters are undoubtedly similar in behavior to qualitative genes. Since the latter exhibit dominance almost universally the same is true of the former; and the mere fact that quantitative character data seem to fit a logarithmic curve does not necessarily rule out dominance.

Houghtaling (8), working with cell number and cell expansion in the tomato fruit, came to the conclusion that a geometric progression should be involved in the inheritance of size of fruit. Sinnott (25), MacArthur and Butler (18), and Charles and Smith (2), from a study of the means and frequency distribution of the parents and F_1 and F_2 generations, interpreted the results from size inheritance studies on the basis of a geometric progression. Rasmusson's (23) interaction hypothesis is based on the assumption that the effect of a certain factor may be smaller the greater the number of factors acting in the same direction. Powers (21), working with habit of growth in *Triticum*, and Rasmusson (23), working with earliness of maturity in *Pisum*, obtained data that would support the interaction hypothesis. Powers (22) found that in general the nature of the interaction of the factors affecting weight of seed per plant, number of spikes per plant, height of plant, and length of awn in segregates from hybrids involving varieties of *Hordeum vulgare* was such that the effect of certain genes was larger the greater the number acting in the same direction. From this review of literature it may be concluded that at least four types of interactions of the genes, as measured by end products, exist. One would be such that the effects, as measured by end results, would be less than arithmetically cumulative; another would be such that the effects would be arithmetically cumulative; another would be such that the effects would be somewhat more than arithmetically cumulative; and the last would be such that the effects would be geometrically cumulative.

The fact that the values for the first-order interactions (table 10) are statistically significant shows that the genes affecting number of locules do interact, and the differences listed under alleles and generations in table 10 show the nature of the interaction, as, for example, the interaction *Gg* \times *Oo^{ob}*, of which the values 0.71 and 1.02 are due to the increased differences between the alleles within the homozygous

recessive genotypes. Likewise, the interactions $Gg \times$ generations and $Oo^{ob} \times$ generations, with the values 0.42 and 0.73 and the values 0.44 and 0.75, respectively, are due to the increased differences between the generations within the homozygous recessive genotypes. It will be remembered that the genes favorable to an increase in number of locules are linked with the recessive marker genes. Then, it can be concluded that the nature of the interaction is such that the genes favorable to an increased number of locules gave greater differences over their alleles in combination with genes for a higher number of locules than they did in combination with genes for a lower number of locules. In fact, the nature of the interactions of the genes was such as to be geometrically cumulative.

It is not possible to predict the exact number of gene pairs involved in differentiating number of locules; but the rather consistent increase in locule number from that of the Red Currant parent to that of the Johannisfeuer parent and the data, already presented, showing that at least three gene pairs affecting locule number must be segregating during sporogenesis of the F_1 generation of the cross Johannisfeuer \times Red Currant make it appear that the parents differ by a large number of genes.

SUMMARY AND CONCLUSIONS

Formulas were developed for determining the theoretical effect of double crossing over and dominance upon the differences between the means of the genotypes involved in inheritance of quantitative characters. These formulas apply only under the conditions stipulated in the text.

A gene having an effect upon a quantitative character and located between the loci of the two marker genes will have an influence upon both regions of the chromosome, and, if the linkage between the two marker genes is sufficient to prevent double crossing over, the summation of the effect for the two regions will include the total effect of the gene pair influencing the quantitative character. However, in making the comparison both regions should be taken into consideration simultaneously; for example, $ggo^{ob}o^{ob} - GgOo^{ob}$.

By means of the three-point method of analysis in those cases in which double crossing over does not occur in the section of the chromosome delimited by adjacent marker genes, it is possible to determine the individual effect of regions of the chromosome upon the quantitative character under investigation. However, the nature of possible interactions between genes closely linked and located within any one region cannot be readily determined.

A region of a chromosome is designated by a pair of marker genes such as Gg , and a section of a chromosome by two adjacent pairs of marker genes such as $Gg-Oo^{ob}$. As previously pointed out by Powers (22), the regions are not fixed but vary in length according to the positions of the chiasmata; but, as has been shown, their effects can be determined and logically summed by using the three-point method of analysis. The same is true for the two-point method of analysis, if only two different regions of the chromosome are being studied.

In the data obtained from the progeny resulting from backcrossing the F_1 generation to the parent recessive for the marker genes, neither

the effects due to double crossing over nor the effects due to dominance are confounded with possible interactions. Likewise, effects of double crossing over and dominance are not confounded with possible interactions in data collected from an F_2 generation, but the effects due to recombination of gametes containing certain single cross-overs are confounded with the interactions. Therefore, tables such as 6 and 8, giving the theoretical values, aid materially in an interpretation of the data.

Double crossing over decreases the proportion of the effect due to the gene tending to increase the quantitative character that is measured. Likewise, the measurable proportionate effect decreases with a decrease in the degree of dominance.

In a study involving number of locules for the segregates of the cross *Lycopersicon esculentum* var. *Johannisfeuer* \times *L. pimpinellifolium* var. *Red Currant*, the combined effect of the genes tending to increase the number of locules per fruit for the Gg and Oo^{ob} regions of the chromosome was 3.87 locules per fruit for the progeny obtained by backcrossing the F_1 generation to the parent recessive for the marker genes and the difference $ggo^{ob}o^{ob} - GgOo^{ob}$ was 2.70 locules per fruit for the F_2 generation.

The interactions $Gg \times Oo^{ob}$, $Gg \times$ generations, and $Oo^{ob} \times$ generations were statistically significant. The nature of the interactions was such that the effect of the genes tending to increase number of locules per fruit was geometrically cumulative for "between means" of genotypes and for "between means" of generations for the different genotypes. These findings are in accordance with those reported by Powers (22) for the nature of the interaction of factors affecting weight of seed per plant, number of spikes per plant, height of plant, and length of awn in segregates from hybrids involving varieties and species of *Hordeum*.

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EFFECT OF MOISTURE, FERTILITY, AND FERTILIZER PLACEMENT ON ROOT ROT OF CANNING PEAS IN WISCONSIN¹

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INTRODUCTION

The importance of high soil fertility in the reduction of damage to peas caused by *Aphanomyces euteiches* Drechsler was first pointed out at the New Jersey Agricultural Experiment Station in 1928 (4, Rept. 49).³ In subsequent reports (2; 4, Repts. 51, 56, 57; 5; 6; 7) these studies, which were carried out by Haenseler, repeatedly confirmed the earlier findings and showed that the mineral nitrogenous components of the fertilizer mixtures used were the most influential ones. The amounts of fertilizer applied were usually from 1,000 to 2,000 pounds per acre. Recently Geach in Australia showed in pot experiments in the greenhouse a measurable reduction in the amount of root rot in peas when nitrogenous fertilizers were added to infested soil.

The present paper reports the results of similar studies conducted in Wisconsin, a preliminary report of part of which has already been made (8). Root rot is most destructive in this State on Colby silt loam, a soil of high water-holding capacity. The disease is, of course, most serious in seasons when rainfall is plentiful during May, June, and July in accordance with the influence of soil moisture on the disease demonstrated by Jones and Drechsler (3). Since the usual fertilizer applications in this section are much smaller (usually 200 to 500 pounds per acre) than in New Jersey it became of interest to know whether such applications have any appreciable effect on the disease, and whether any relief from the latter may be expected without resorting to larger and otherwise unnecessary rates.

EXPERIMENTAL RESULTS

GREENHOUSE EXPERIMENTS

Colby silt-loam soil from a root-rot-infested field near Owen, Wis., was placed in a greenhouse bench to a depth of about 5 inches. Half of the area was left unfertilized while the remainder was supplied with 4-16-4 fertilizer at the rate of 500 pounds per acre, spread broadcast and worked into the upper 3 inches of the soil. Seed of three varieties—Bruce, Alaska, and Surprise—were planted in rows. The fertilized and unfertilized areas were further divided each into two equal portions. In one of each the soil was moistened only enough to permit germination and fair growth; in the other the soil was kept at a relatively high moisture level by frequent watering. Part of the plants were removed at 32 days and the remainder at 39 days.

¹ Received for publication April 21, 1939.

² Died September 14, 1939.

³ Italic numbers in parentheses refer to Literature Cited, p. 590.

After the roots and lower stems were examined the plants were sorted into three groups—healthy or slight, moderate and severe—according to the degree of root rot.

When the results from the dry and the moist soils are compared (table 1) it is clear that the disease was almost completely suppressed in the former. This is in accord with the common observation on Colby silt loam in central Wisconsin where root rot is difficult to find in dry seasons and is usually very destructive in moist seasons. The reduction of the disease in fertilized moist soil as compared with that in unfertilized moist soil was very striking, showing the same trend of result as that reported on New Jersey soils.

TABLE 1.—*The effect of soil moisture and fertility on the occurrence of root rot in three varieties of peas grown in the greenhouse*

Fertilizer treatment	Variety of pea	Interval after planting	Plants in various disease groups in—					
			Dry soil			Moist soil		
			Healthy to slight	Moderate	Severe	Healthy to slight	Moderate	Severe
		Days	Percent	Percent	Percent	Percent	Percent	Percent
None.....	Bruce.....	32	100	0	0	29	0	71
		39	100	0	0	2	5	93
	Alaska.....	32	98	2	0	44	29	27
		39	100	0	0	0	0	100
	Surprise.....	32	95	0	5	37	8	55
		39	100	0	0	3	1	96
Average.....			98.8	.3	.8	19.2	7.2	73.7
4-16-4 500 pounds per acre.	Bruce.....	32	97	0	3	97	0	3
		39	100	0	0	93	0	7
	Alaska.....	32	100	0	0	79	21	0
		39	100	0	0	97	0	3
	Surprise.....	32	100	0	0	87	13	0
		39	100	0	0	89	1	10
Average.....			99.5	0	.5	90.3	5.8	3.8

Two successive plantings were made on the same soil without further fertilization. For these tests all the soil was maintained at a high moisture level. Five other canning varieties were included in one or the other of these trials. The results (table 2) have the same trend for each of the varieties. In all cases more than 60 percent of the plants were severely diseased in the unfertilized soil, with an average of 79 percent; in only 2 out of 10 instances were more than 30 percent of the plants severely diseased in the fertilized soil, while the average was 19 percent. Thus one fertilizer application, under greenhouse conditions, was sufficient to reduce markedly the damage from root rot in three successive crops of peas, each grown to about the first-blossom stage.

It is well at this point to describe the appearance of fertilized and unfertilized plants at the end of the experiments. Representative individuals of the variety Bruce from each treatment in moist soil in the first experiment are shown in figures 1 and 2.

The striking difference in the size of plants shown in figure 1 is obviously more than fertilizer effect. The comparison of root systems



FIGURE 1.—Peas of the variety Bruce grown in the greenhouse on root-rot-infested Colby silt-loam soil, collected 32 days after planting: A, Plants grown on a portion of the soil that had been treated with 500 pounds per acre of 4-16-4 fertilizer; B, plants from the unfertilized soil. (See fig. 2.)

in figure 2 illustrates the difference in disease development. Fertilized plants are not in any sense free from infection. In the unfertilized plants the degree of invasion and rootlet destruction is much more

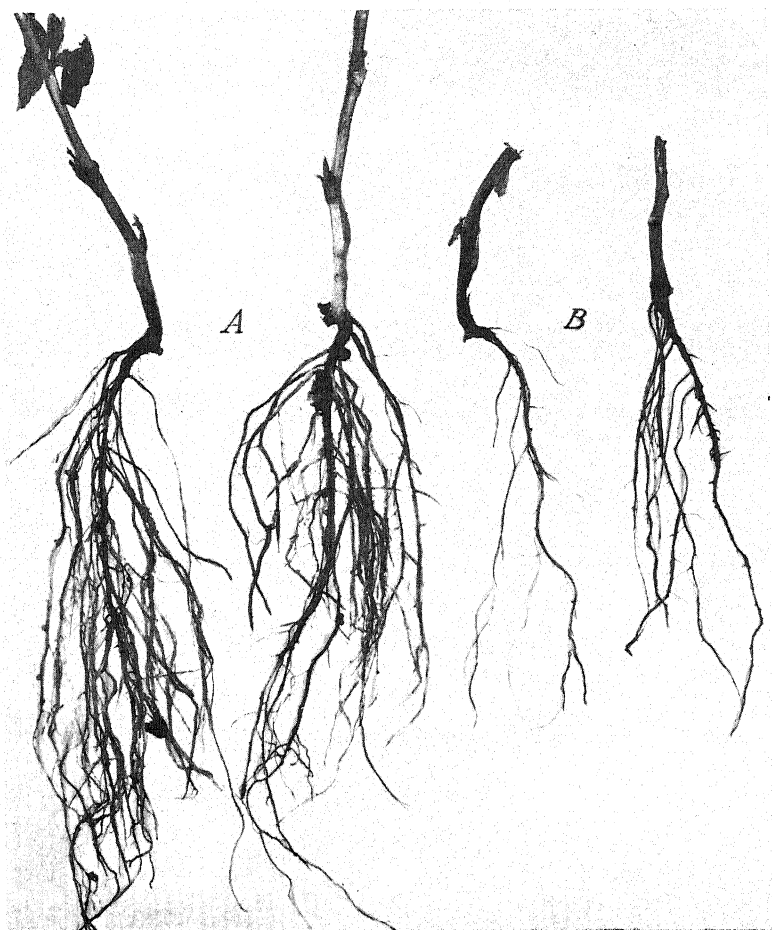


FIGURE 2.—Representative plants from the fertilized (*A*) and unfertilized (*B*) groups shown in figure 1. Note the relative severity of root rot in the two lots.

severe, while the progress of the fungus into the lower stem and the decay of the cortex in that region are also greater. It is difficult to estimate the extent of replacement of rootlets in the two differently treated plants. In the main the basic difference seems to lie in the fact that invasion by the fungus is much less aggressive in the roots of the fertilized plants because of the greater resistance of these plants. There seems to be no question of this fact insofar as the subterranean portions of the stems are concerned.

Fertilizer trials in the field on Colby silt loam were begun in 1932. During this and succeeding seasons up to and including 1937 little was accomplished in the way of tangible results because of the general

lack of severe root rot. This was correlated, of course, with the relatively low precipitation during the critical periods for root-rot development. In 1938, very favorable weather for root rot occurred. In heavily infested fields damage was severe. The relation of fertilizer application to severity of the disease was brought out very definitely in fertilizer trials conducted on Colby silt loam at the Branch Experiment Station at Marshfield, Wis.

TABLE 2.—*The effect of fertilizer upon the occurrence of root rot in several varieties of peas grown in the greenhouse*

Variety	Number of planting following treatment	Severely diseased plants in—		Variety	Number of planting following treatment	Severely diseased plants in—	
		Unfertilized soil	Fertilized soil			Unfertilized soil	Fertilized soil
		Percent	Percent			Percent	Percent
Alaska.....	{Second	69	11	Perfection	Second	95	19
	{Third	66	20	Prince of Wales.....	Third	63	6
Surprise.....	{Second	73	24	Wisconsin Early	do.....	65	35
	{Third	90	34	Sweet.....			
Brace.....	Second	97	9	Average		79	19
Green Admiral.....	do.....	80	5				
Ashford.....	do.....	87	28				

FIELD EXPERIMENTS

The Marshfield experiment was one of a series, all of which were identical in plot design, conducted by the junior author, on five soil types in various locations in Wisconsin. Root rot was noticeable only in the plot at Marshfield. The largest part of the area used for this experiment had been used for peas 3 years previously (1935). Although root rot was not serious in that year on this field, it should be borne in mind that weather conditions in 1935 were not favorable to the disease. The remainder of the 1938 experimental area had not grown peas for a much longer period. Alaska peas were planted on May 13. At this time the upper 6 inches of soil contained 35 percent of moisture, an amount much above normal when compared with previous moisture records secured at planting time at the Marshfield station. With frequent and often heavy rains following the time of planting (2.54 inches during the first 7 days after planting) root rot appeared early, and at blossom time serious damage was already evident. It was clearly seen at this date that root rot was much more severe in the part of the field that had grown peas in 1935.

When the plants were in the early-blossom stage random samples were taken from each of the plots. The average height of the plants was recorded and after the soil had been washed from the roots carefully, the percentage of severely damaged individuals was determined. In table 3 are presented the data obtained together with the order of arrangement and the various treatments. Two formulas of fertilizer, one with nitrogen (2-12-6) and one without (0-20-10) were used on duplicate plots. The first of these was used at two rates (200 and 300 pounds per acre). All fertilizer was applied at the time of planting with a combination fertilizer-grain drill, by means of which the material could be placed (1) in the drill row directly with the seed, (2) in a furrow $1\frac{1}{2}$ inches to the side of and at the same level as the seed, or (3) in a shallow furrow directly over the seed, from which it was

separated by about an inch of soil. Plots 15, 16, 17, and 18 were in that part of the field on which peas had not been grown for several years, and in which root rot was least severe.

TABLE 3.—*The effect of fertilizer applications on vine height and on severity of root rot of Alaska peas grown on Colby silt loam at Marshfield, Wis., 1938*

Plot No.	Fertilizer treatment			Condition at blossom period	
	Formula	Amount per acre	Placement with relation to seed	Average vine height	Severely diseased plants
		<i>Pounds</i>		<i>Centimeters</i>	<i>Percent</i>
1.....	2-12-6	200	With.....	36	27.3
2.....	2-12-6	200	Side.....	35	17.9
3.....		0		34	76.5
4.....	2-12-6	300	With.....	39	21.4
5.....	2-12-6	300	Side.....	35	44.1
6.....	2-12-6	300	Over.....	40	30.8
7.....		0		28	84.4
8.....	0-20-10	200	Side.....	30	63.6
9.....	0-20-10	200	With.....	33	61.1
10.....	2-12-6	200	Side.....	30	71.8
11.....	2-12-6	200	With.....	41	29.4
12.....		0		30	55.6
13.....	0-20-10	200	Side.....	30	46.7
14.....	0-20-10	200	With.....	34	41.7
15 ¹	2-12-6	300	do.....	42	8.3
16 ¹	2-12-6	300	Side.....	39	12.5
17 ¹		0		38	14.3
18 ¹	2-12-6	300	Over.....	40	7.7
Average.....	2-12-6	200-300	With.....	38.7	26.0
	2-12-6	200-300	Side.....	33.3	44.6
	0-20-10	200	With.....	33.5	51.4
	0-20-10	200	Side.....	30.0	55.2
		0		30.7	72.2

¹ Since these plots were on soil relatively low in root rot they are not included in the averages.

Where the same fertilizer treatment was used with and at the side of the seed it is to be noted that in every instance the plants with fertilizer in the furrow were the tallest at this stage. In most instances both were taller than the nearest unfertilized plants. When the percentage of severe root rot is considered it is evident that it was practically always lower in the fertilized plots than in the nearest checks. The adjoining plots of the same treatment usually showed less severe root rot when the fertilizer was applied with the seed than when it was applied at the side of the seed. The application of fertilizer over the seed was not included in all of the comparisons, but where it was so applied the effects were intermediate between the application with and at the side of the seed. There are indications that the nitrogenous fertilizer was more effective than the nonnitrogenous when the 200-pound rate of 2-12-6 and 0-20-10 are compared. This differential effect confirms the observations of Haenseler (4, Rept. 56). The fact that, when relatively low rates are used, the benefits are greatest when the fertilizer is immediately available to the plant does not seem to have been emphasized previously. Obviously when the fertilization program requires 1,000 to 2,000 pounds per acre, application with the seed is out of the question because of the danger of seedling injury. With 300-pound rates of formulas relatively low in nitrogen under Wisconsin conditions, however, the application of fertilizer with the seed has not been observed to be

injurious. In fact, better stands are secured more often on the loams and silt loams where moderate applications are applied with the seed.

The appearance of fertilized and unfertilized plants removed from the soil at blossom time was similar to that already observed in the greenhouse and illustrated in figures 1 and 2. The relative height of vines of plants from plots 3, 4, and 5 is shown in figure 3, while the condition of the root systems with respect to root rot in representative plants from each of these plots is illustrated in figure 4. The striking difference between the severity of root rot in the roots from plants grown with fertilizer in the row and at the side of the row is in accord with the actual counts recorded in table 3.

At the canning stage random samples were again taken from plots 3, 4, and 5. Representative plants from each are shown in figure 5. In these plots as well as in the remaining ones the effect of root rot on yield was obvious and the influence of the type of fertilizer placement on the degree of recovery of plants from the disease was a measurable one. In studies of this sort it is difficult to differentiate the direct effect of fertilizer on plant growth and yield from that of the effect on plant resistance. Some measure of this may be taken in the analysis of disease severity already presented. It was fortunate, however, that the plot design was repeated exactly in four other locations in none of which root-rot damage could be found. It is thus possible to get some measure of the indirect effect of fertilizer on yield through disease control by comparing the yield records at all five locations. These are summarized for each treatment in table 4. For each treatment there were duplicate plots at each location. The data from the non-root-rot plots were treated for analysis of variance and the minimum difference, at the 5-percent level, required for significance between the averages of yields for each treatment at four locations is included in the table.

The effectiveness of various fertilizer placements on the non-root-rot plots is of particular interest. In every treatment, except that of 300 pounds of 2-12-6 on the Knox silt loam, the results show the superiority of applying fertilizer down the same drill spout with the seed. The increased yield secured by applying fertilizer in this manner was especially marked on two of the four soil types, namely, the Carrington silt loam and the Superior clay loam. The average of the plots on the Superior clay loam gave a 21-percent increase in yield of fertilized over the unfertilized, and in the case of the Carrington soil it was 27 percent. Similarly the averages for the plots receiving the side application gave increases of 6 and 17 percent, respectively. The averages for each of the treatments for the four different soil types, as indicated in table 4, show increases ranging from 13.7 to 22.2 percent over the unfertilized where the fertilizer application was made with the seed, whereas when it was applied at the side of or above the seed the increase in no case reached 10 percent.

In general the same relative results were maintained in the root rot soil with the additional important fact that the spread between the "side" and "with" application was of much greater magnitude. The highest percentage increase (248.5 percent) was secured where 300 pounds of 2-12-6 were applied with the seed.

While the percentage of increase in yield in fertilized over unfertilized plots was much greater in the root rot soil than in the non-root-rot soil, the actual yields even on the best plots (300 pounds of

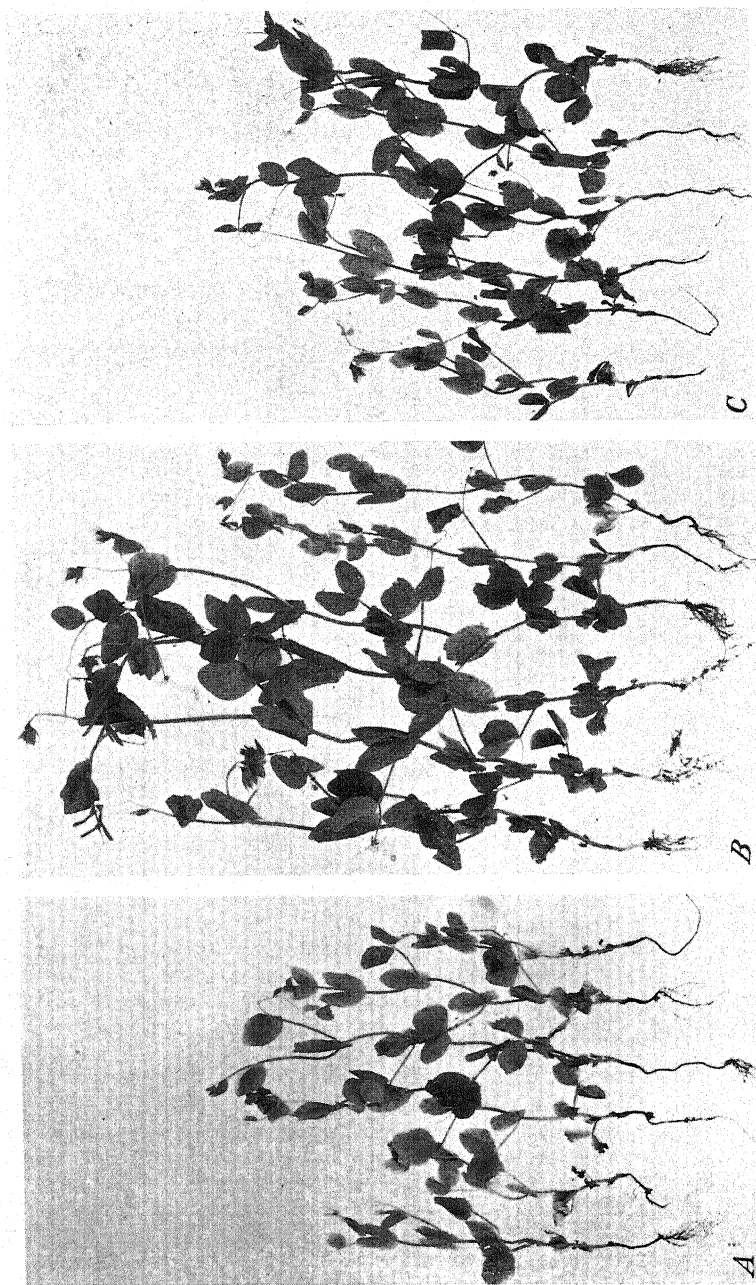


FIGURE 3.—Representative plants taken at the blossom stage from plots 3, 4, and 5 (table 3) of the Marshfield experiment, 1938, on root-rot-infested soil: A, No fertilizer; B, 300 pounds per acre of 2-12-6 with the seed; C, 300 pounds per acre of 2-12-6 at side of seed. Note the relatively greater height of plants from the plot in which fertilizer was applied with the seed. (See fig. 4.)

2-12-6, "with") was much lower than on the plots on which the soil was free of root-rot. It appears that quickly available nutrients placed where root development occurs in its early stage makes it

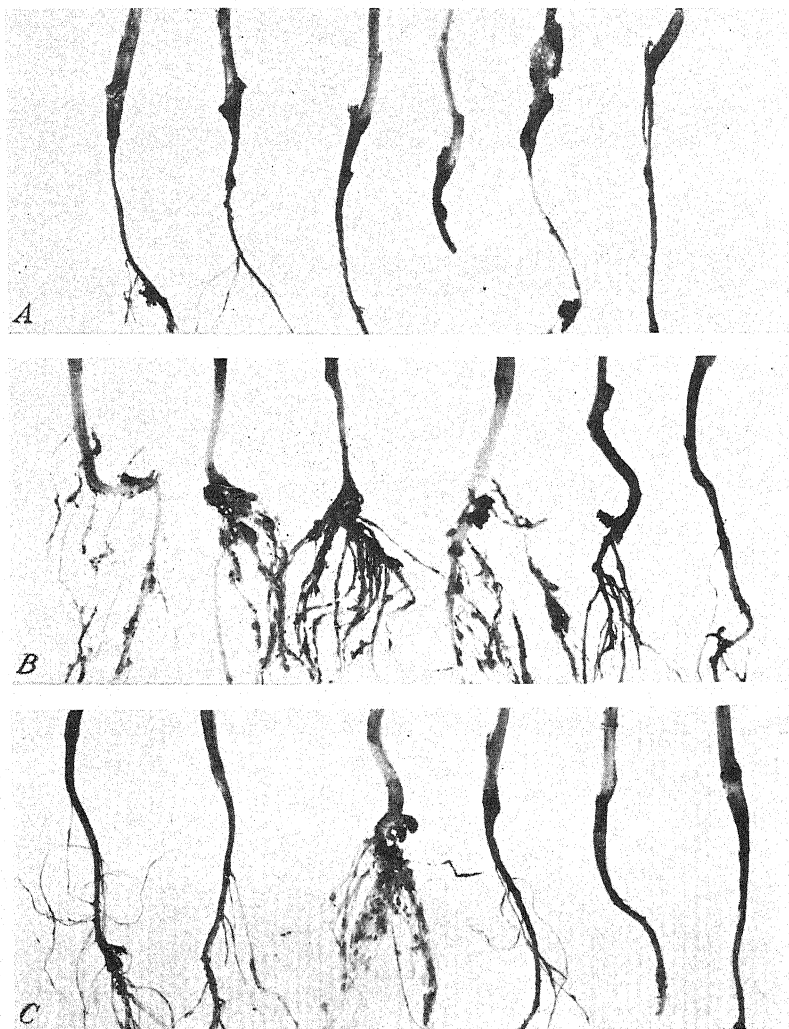


FIGURE 4.—Roots of plants taken at blossom stage from the same plots as those shown in figure 3: A, No fertilizer; B, 300 pounds of 2-12-6 with the seed; C, 300 pounds of 2-12-6 at side of seed. Note that in the unfertilized soil the disease has resulted in decay of most of the fibrous root system as well as the cortical tissue of the lower stem. While the disease is present in the fertilized plants it has been less destructive and many healthy roots are still functional, particularly where the fertilizer was applied with the seed. (See fig. 5.)

possible for the plant to develop greater resistance to the invasion of the root-rot organism. It is evident also that fertilizer placement is an important factor in developing such resistance. Then too, the much greater increase on the plots receiving nitrogen in addi-

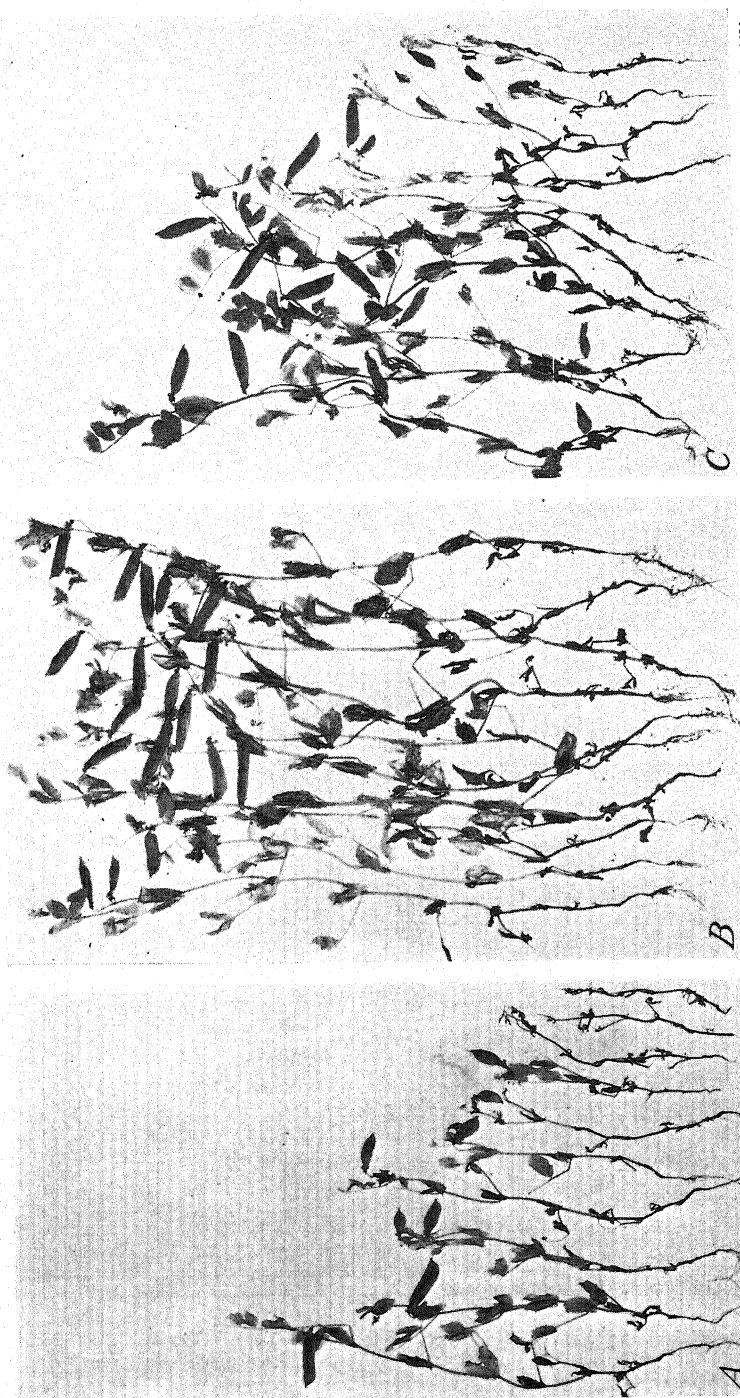


FIGURE 5.—Representative plants collected at canning stage from the same plots as those illustrated in figures 3 and 4: A, No fertilizer; B, 300 pounds of 2-12-6 with the seed; C, 300 pounds of 2-12-6 at side of seed. Note the superiority of the plants which were fertilized by placement with the seed. Compare with the yield data given in table 4.

tion to phosphate and potash brings out the importance of nitrogen in root-rot control as already emphasized at the New Jersey station (4, Rept. 56).

TABLE 4.—*The relative yield of peas on root-rot-infested soil (Colby silt loam) and on four other types that were not infested, when various fertilizer treatments were made at the time of sowing, 1938*

Fertilizer treatments			Yield of peas per acre on the indicated soil types at stated locations in Wisconsin							
			Non-root rot soils						Root-rot soil, Colby silt loam near Marshfield	
			Miami silt loam near Columbus ¹	Car-rington silt loam near Fall River ¹	Superior clay loam near Osh-kosh ²	Knox silt loam near Du-rand ¹	Average of non-root rot soils			
Formula	Amount per acre	Place-ment with re-lation to seed					Yield	Increase over control	Yield	Increase or decrease over control
	Pounds		Pounds	Pounds	Pounds	Pounds	Pounds	Percent	Pounds	Percent
0-0-0			2,518	2,457	3,040	1,535	2,388		241	
	200	With	2,702	3,230	3,390	1,536	2,715	+13.7	509	+111.2
	200	Side	2,484	2,870	2,935	1,440	2,432	+1.8	309	+28.2
2-12-6	300	With	2,770	2,970	3,820	1,593	2,788	+16.8	840	+248.5
	300	Side	2,508	2,910	3,395	1,632	2,611	+9.3	405	+68.0
	300	Over	2,624	2,790	3,340	1,561	2,579	+8.0	512	+112.4
0-20-10	200	With	2,948	3,165	3,860	1,702	2,919	+22.2	352	+46.5
	200	Side	2,698	2,830	3,360	1,613	2,625	+9.9	207	-14.1
Difference required for significance at the 5-percent level							230			

¹ Alaska variety used.

² Wisconsin Perfection variety used.

³ Estimated value; 1 degree of freedom lost in error.

SUMMARY

A study was made of the relation of fertilizer treatment to the severity of rot (*Aphanomyces euteiches* Dreschler) in Colby silt-loam soil.

Experiments in the greenhouse with several varieties of canning peas showed marked control of the disease in three successive crops grown on soil fertilized with one application of 4-16-4 fertilizer at the rate of 500 pounds per acre.

In field experiments conducted on five soil types in five locations in Wisconsin the comparative productiveness of peas and the relative severity of root was studied when fertilizer was applied in various ways. In non-root-rot soils with 200 pounds and 300 pounds per acre, the greatest increases in yield, averaging 13.7 to 22.2 percent above that of the untreated plots, were secured when the fertilizer was applied down the same drill spout with the seed. When the fertilizer was applied about 1½ inches to the side of and at the same level as the seed the increases averaged less than 10 percent.

In general the same relative results were secured in the root-rot-infested soil, with the additional important fact that the percentages of increases in yield were much greater. The difference in the plots where fertilizer was placed at the side of the seed varied from a decrease of 14 percent to an increase of 68 percent over the control, while in the cases where the fertilizer was applied with the seed the increases were from 46 to 248 percent.

It appears that quickly available nutrients placed in amounts of 200 to 300 pounds per acre in the furrow with the seed made it possible for the plants to develop greater resistance to root rot. Much greater reduction of root rot and increases in yield were secured when 2 percent of readily available nitrogen was included in the fertilizer than when it was omitted.

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RELATION OF TEMPERATURE AND MOISTURE TO NEAR-WILT OF PEA ¹

By WALTER J. VIRGIN, formerly *research assistant*, and J. C. WALKER, *professor of plant pathology, Wisconsin Agricultural Experiment Station*

INTRODUCTION

Practically complete control of the wilt disease (*Fusarium orthoceras* App. and Wr. var. *pisi* Linford), which was formerly very destructive to the pea crop of Wisconsin, eastern Washington, and parts of Maryland, has now been accomplished by the development and general adoption of suitable wilt-resistant varieties. The description of this disease and the causal organism has been given in detail by Linford.² More recently another disease of pea, known as near-wilt, has been distinguished in the same areas and even over a wider geographical range. This malady and the causal pathogen (*F. oxysporum* Schlecht. f. 8 Snyder) have been described by Snyder and Walker.³ While the near-wilt disease appears to be much more widespread in the United States than wilt, it has never become so acutely destructive in the regions in which it has become established. In fact it is usually to be found on occasional plants, widely scattered in the field, and only uncommonly does it destroy a majority of the pea plants within a given area. Whether this is due to the relatively poor capacity of the near-wilt organism to establish itself as abundantly and rapidly in favorable soils as does the wilt fungus remains to be determined, but it does appear from field observations that the former organism occurs over a wider range of soil types than the latter.

Symptoms of the two diseases resemble one another in many respects. The foliage becomes yellow with both and the leaflets and stipules of the plants may curve downward and inward, while at high temperatures they may quickly wither from the base of the plant upward. Stunting and rapid loss of turgidity of the wilt disease are less common with near-wilt. On the other hand, unilateral development of symptoms is more common with the latter disease. The pathogen, traveling rather rapidly up the stem, in one or a few vascular bundles, affects leaves and stipules in its path from base to tip. This localized effect commonly extends to one stipule in a pair, to leaflets on one side of the petiole, and even to the portion of the leaf lamina on one side of the midrib. In general the affected vascular system is orange to deep red in color in contrast to the lighter orange characteristic of bundles affected by wilt. This color difference, together with the fact that the near-wilt organism and the discoloration following it travel up the stem often to the growing tip while in wilt they seldom advance above the fifth node, is a very useful means of distinguishing the two diseases.

¹ Received for publication May 22, 1939.

² LINFORD, MAURICE B. A *FUSARIUM* WILT OF PEAS IN WISCONSIN. Wis. Agr. Expt. Sta. Res. Bul. 85 44 pp., illus. 1928.

³ SNYDER, W. C., and WALKER, J. C. *FUSARIUM* NEAR-WILT OF PEA. Zentbl. f. Bakt. [etc.] (II) 91: 355-378, illus. 1935. See p. 373.

The purpose of the investigation reported in this paper was to study the effect of certain enviroanal factors, especially temperature and moisture, upon the expression of near-wilt symptoms. This seemed to the writers to be particularly important in view of the attention being given to the possibility of developing near-wilt-resistant varieties suitable for commercial production.

EXPERIMENTAL RESULTS

TEMPERATURE RELATIONS OF THE NEAR-WILT ORGANISM

The relation of temperature to the growth of *Fusarium oxysporum* f. 8 in pure culture was studied in plate cultures of potato-dextrose agar which had been adjusted to pH 6.2. The isolate was from typically diseased plants in the field and its identity was confirmed by

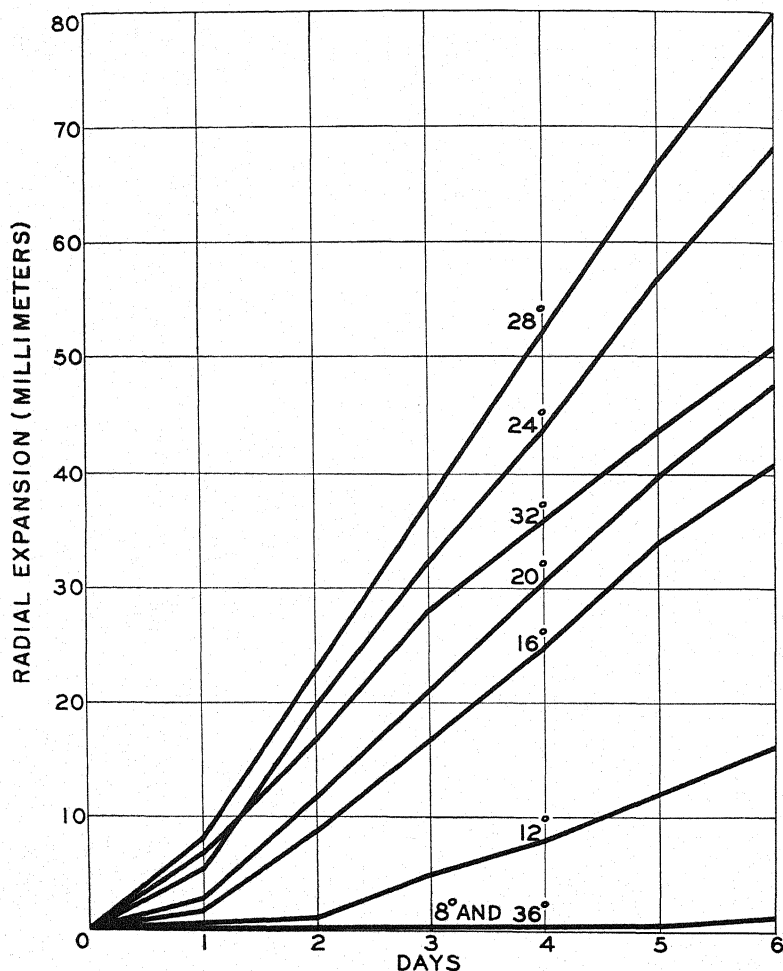


FIGURE 1.—Daily radial expansion of *Fusarium oxysporum* f. 8 on potato-dextrose agar plates incubated at various temperatures.

W. C. Snyder of the University of California. Five inoculated plates were placed in controlled incubators at each of the following temperatures: 4°, 8°, 12°, 16°, 20°, 24°, 28°, 32°, 36° C. After colonies had become established the radial growth of each plate was recorded every 24 hours until the thallus covered the entire plate. No measurable growth occurred at 4°. The average daily radial growth is plotted in a graph for each of the other eight temperatures (fig. 1). Since each of these graphs is practically a straight line it may be assumed that little or no staling material which retarded growth was produced by the fungus. Rates of growth at 8° and 36° were approximately the same. Most rapid radial expansion occurred at 28°. These results agree in general with those reported for other forms of *F. oxysporum*.⁴ The maximum, minimum, and optimum temperature for growth as measured by this method came relatively close to coinciding with those of the wilt fungus.⁵ It may be pointed out here, however, that whereas the optimum temperature for wilt development is much lower than that for its growth on agar, the data to be presented later in this paper show that the optima are closer in the case of near-wilt.

RELATION OF VARIETY TO DISEASE-EXPRESSION

During the course of this investigation a study of varieties of peas (*Pisum sativum* L.) in relation to near-wilt was carried out in both field and greenhouse. It was noted that, generally speaking, the symptoms of near-wilt were slower to appear than those of wilt. Furthermore their development in the case of near-wilt varied with the variety when a number of varieties were grown in the same location. In most cases there was a distinct coincidence under field conditions in southern Wisconsin and in the greenhouse at Madison, Wis., between appearance of near-wilt and the beginning of the blossoming period. Thus early-blossoming varieties showed the disease early while in late-blossoming varieties the appearance of symptoms was delayed. A common example of this is the contrast between the Alaska, a variety which blooms and matures early, and Alderman, a late variety. Under optimum conditions for the disease the plants of Alaska are commonly dead from near-wilt before any signs of disease are shown by the Alderman plants, although the latter eventually succumb. In certain varieties which are very susceptible the plants may wilt before blossoming has occurred. The differential effect of varieties on the progress of the disease has been taken into account, therefore, in the present study of the effect of external environment upon near-wilt.

TEMPERATURE RELATIONS OF THE DISEASE

Soil-temperature experiments were carried out in the greenhouse in Wisconsin tanks. The pathogen was increased on a medium of corn meal and sand which was incorporated with soil that had been collected from an uncultivated wood lot and autoclaved for 5 hours at 15 pounds pressure. Since the volume of inoculum used was very small in relation to that of the soil, a period of 2 months was allowed to elapse during which the mixture was stirred and watered occasionally in order

⁴ GOSS, R. W. RELATION OF ENVIRONMENT AND OTHER FACTORS TO POTATO WILT CAUSED BY *FUSARIUM OXYSPORUM*. Nebr. Agr. Expt. Sta. Res. Bul. 23, 84 pp., illus. 1923.

JOHNSON, JAMES. *FUSARIUM WILT OF TOBACCO*. Jour. Agr. Res. 20: 515-535, illus. 1921.

⁵ See footnote 2.

to permit the organism to become uniformly established. It was then placed in galvanized iron cans which were set into the respective tanks. A quantity of sterilized, uninoculated soil from the same source was placed in other cans to serve as controls. For comparative purposes soil naturally infested with the wilt organism was placed in still other cans.

Five tanks were adjusted to 16°, 20°, 24°, 28°, and 30° C., respectively. In each tank were placed four cans of near-wilt-infested soil, two cans of wilt-infested soil, and 2 cans of uninoculated soil. The moisture content of the soils was approximately 60 percent of their water-holding capacity and the moisture was kept fairly constant by frequent weighings of the cans and replacement of the water lost by evaporation and transpiration.

In each tank two cans of near-wilt soil and one can of uninoculated soil were planted with seeds of Early Kay, a wilt-resistant and somewhat near-wilt-resistant variety of pea, and a similar set was planted with Wilt Resistant Perfection, a wilt-resistant, near-wilt-susceptible variety. The wilt soil (two cans in each tank) was planted with wilt-susceptible Perfection. Ten plants were grown in each can.

Plants were removed and counted as soon as they were permanently wilted and the identity of the disease was confirmed by recovering the respective fungus from each plant. A wilt index or near-wilt index was calculated for each can or duplicate cans by adding the number of days from sowing to wilting for each plant and dividing by the total number of plants, the index being the average number of days for the plants to reach the wilt stage. Thus the more rapid the development of the disease the lower the index. The data from this experiment are given in table 1.

Since the plants in the uninoculated soil all remained healthy they are omitted from the table. The most rapid development of wilt was at 20° C. which is in accord with the report of Linford⁶ that the optimum was between 21° and 22°. It is to be seen, however, that the disease development was nearly as rapid at 24°, 28°, and 30° as at 20° while a definite retardation occurred at 16°. In the case of Wilt Resistant Perfection growing on near-wilt soil the most rapid disease development was at 24°, although there was little difference at this temperature from 20° and 28°. Distinct retardation occurred at 16° and 30°. It is evident that the disease was limited more definitely at 30° than was wilt, while at 16° they were both distinctly retarded.

TABLE 1.—Indices of wilt and near-wilt in Wilt Resistant Perfection (wilt-resistant, near-wilt-susceptible), Perfection (wilt-susceptible), and Early Kay (wilt-resistant, near-wilt-resistant) pea varieties at various soil temperatures

Variety	Wilt index (days) at soil temperature of—					Near-wilt index (days) at soil temperature of—				
	16° C.	20° C.	24° C.	28° C.	30° C.	16° C.	20° C.	24° C.	28° C.	30° C.
Perfection.....										
Wilt Resistant Perfection.....	1 39	25	27	28	2 27	3 64	46	44	46	4 55
Early Kay.....						(5)	(5)	62	63	68

¹ 4 plants alive at the end of the experiment.

² 1 plant alive at the end of the experiment.

³ 2 plants alive at the end of the experiment.

⁶ See footnote 2.

⁴ 4 plants dead at the end of the experiment.

⁵ No plants dead at the end of the experiment.

⁶ 16 plants living, 4 dead at the end of the experiment.

In addition to being somewhat more restricted in its optimum soil-temperature range, near-wilt was definitely slower in appearing. Thus in the two strains of Perfection it required, on an average, nearly twice as long for all plants to succumb to near-wilt at 20° to 28° C. as was the case with wilt at the same temperatures. The

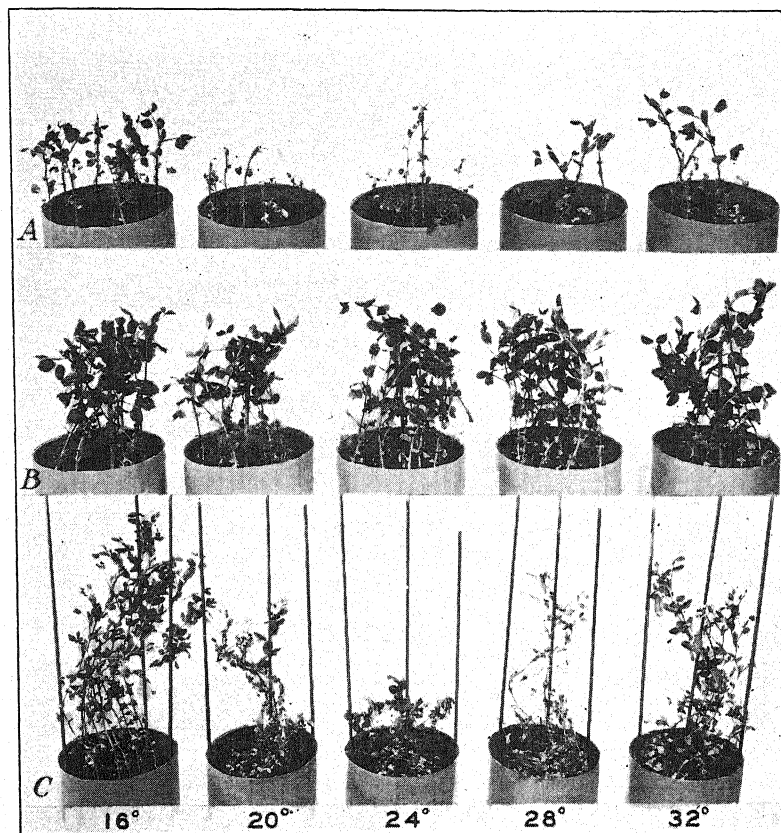


FIGURE 2.—Relation of soil temperature to the development of wilt and near-wilt. *A*, Wilt-susceptible Perfection peas grown in wilt-infested soil for 30 days at the respective constant soil temperatures indicated at the bottom of the figure. *B*, Near-wilt-susceptible Wilt Resistant Perfection plants grown for 30 days in near-wilt-infested soil in parallel series with those in *A*. Note that at this interval the wilt-susceptible plants are all dead or severely diseased at 20°, 24°, and 28° C., while no signs of disease have appeared in the plants on near-wilt soil. *C*, The same series as *B* which has been continued at the respective temperatures for 22 days longer. Note that near-wilt has now developed to a severe degree at 20°, 24°, and 28°, showing that it requires much longer to develop than does wilt, even in optimum environment.

data from Early Kay showed the effect of plant resistance on lengthening the time required for plants to succumb. They showed also that at the higher temperatures the disease eventually affected all plants, even of a variety which shows marked resistance under field conditions. Certain of the plants in this experiment are illustrated in figure 2.

The same test was applied with the inoculated near-wilt soil in a second experiment. Only the most favorable temperatures, 20°, 24°, and 28° C., were included. The two varieties used were Alaska and Horal. The latter, being a late-blooming variety, had shown a longer incubation period in the field and definite evidence of resistance. In a third test Alaska and Wilt Resistant Perfection were run at 16°, 20°, and 24° in naturally-infested near-wilt soil from a field near Winneconne, Wis. The results of these trials are given in table 2. The near-wilt index tended to be lower in the naturally-infested than in the artificially inoculated soil. This is found to be the case when the two Alaska tests in table 2 and the Wilt Resistant Perfection tests in tables 1 and 2 are compared. It may be due to a heavier infection or to the supplementary effect of other organisms which are pathogenic on pea roots, in the naturally infested soil. The same inoculated soil was used several months later for the next experiment (table 3), in which it will be noted that the near-wilt index is about the same for Wilt Resistant Perfection as that in the naturally infested soil of the last experiment. Whether this change is due to the increase in the amount of near-wilt inoculum or to the influence of other organisms with which contamination had occurred cannot be stated.

TABLE 2.—*Near-wilt indices in certain pea varieties in artificially inoculated and in naturally infested soil*

Type of soil infestation	Variety of pea	Near-wilt index ¹ at soil temperature of—			
		16° C.	20° C.	24° C.	28° C.
Artificially inoculated.....	Horal.....	82	80	74
	Alaska.....	46	42	49
Naturally infested.....	Alaska.....	(²)	39	36
	Wilt Resistant Perfection.....	(³)	46	38

¹ The near-wilt index in each case is based on 10 plants.

² At 60 days only 1 plant had wilted.

³ At 60 days only 2 plants had wilted.

It is to be noted that, in accord with what has been pointed out in the previous section, the index was higher in Wilt Resistant Perfection, a late-blooming variety, than in Alaska, an early-blooming variety. This difference was only slight, however, at the optimum. Alaska and Wilt Resistant Perfection are regarded as very susceptible to near-wilt. The comparison between Alaska and Horal brings out again the striking difference in index between an early susceptible and a late resistant variety. Horal is about the same in season as Wilt Resistant Perfection but the near-wilt index of the former is nearly twice that of the latter, discounting the slight discrepancy to be attributed to the difference between inoculated and naturally infested soil. Thus the variety usually highly resistant in the field shows considerable resistance in the greenhouse, but eventually succumbs.

The same artificially inoculated soil used in the above experiments was employed in a study of the relation of air temperature to the development of near-wilt. Three soil temperatures were used—16°, 22°, and 28° C.—at each of three air temperatures—16°, 20 to 22°, and 28°. Wilt Resistant Perfection was planted in three cans at each soil temperature in each air temperature, the total number of plants

in each three-can group averaging about 35. The near-wilt indices secured in this experiment are recorded in table 3. The optimum soil temperature was 28° regardless of the air temperature to which the tops were exposed. At any given soil temperature the differences in rate of disease development between the three air temperatures was not great although the smallest index in each case was at 20°–22°.

TABLE 3.—*Near-wilt indices in Wilt Resistant Perfection peas grown in artificially inoculated soil at 3 soil temperatures in each of 3 air temperatures for 57 days*

Air temperature (° C.)	Near-wilt index at soil temperature of—		
	16° C.	22° C.	28° C.
16°	1 51	38	28
20°–22°	2 47	34	25
28°	3 52	35	27

¹ Based on 8 diseased plants from a total of 37.

² Based on 25 diseased plants from a total of 48.

³ Based on 13 diseased plants from a total of 42.

It may be concluded from these experiments that the soil temperature is much more influential upon near-wilt than is air temperature. While an exact soil-temperature optimum cannot be defined it is evident that constant soil temperatures of 24° to 28° C. usually result in the most rapid development of the disease. Thus the optimum for near-wilt may be regarded as about 5° higher than that for wilt. Temperatures around 16° greatly retard near-wilt regardless of the air temperature. On the other hand, an air temperature of 16° does not appreciably affect the progress of the disease provided the soil temperature is maintained near the optimum. These statements apply particularly to the near-wilt susceptible varieties such as Alaska and Wilt Resistant Perfection. In the second experiment (table 2) there is an indication that the resistant variety, Horal, has a higher optimum than the susceptible variety, Alaska. These studies should be continued with resistant varieties.

RELATION OF SOIL MOISTURE TO THE DISEASE

Soil-moisture studies were conducted in Wisconsin soil-temperature tanks where the temperature variable could be eliminated by running all tanks at 24° C. in a common air temperature of 21° to 22°. Three soil types were used—two infested with near-wilt, one not infested. The infested soils were: (1) A sandy loam from a naturally infested field at Winneconne, already mentioned in connection with the soil-temperature experiments; and (2) a black silt loam which had been artificially inoculated. The noninfested soil was also a silt loam, but was lower in organic matter than the inoculated soil. It was sterilized before use. These soils were each made up into three groups designated as dry, medium-moist, and wet. The water-holding capacity of each soil and the actual moisture content of each group were determined. The percentage of water-holding capacity for each group is given in table 4.

The data from two series, including two susceptible (Alaska and Wilt Resistant Perfection) and two resistant (Kay and Early Kay) varieties are given in table 5. Inasmuch as no disease developed in the noninfested soil no data therefrom are included. The best growth in this soil occurred in the medium-moist lot. There was distinct retardation in growth in the dry soil. In the wet soil, yellowing of the lower leaves was pronounced.

TABLE 4.—*The water-holding capacity of the lots of soil used in the study of the relation of soil moisture to near-wilt*

Description of soil	Moisture as water-holding capacity in—		
	Dry group	Medium-moist group	Wet group
	Percent	Percent	Percent
Naturally infested sandy loam.....	25	47	67
Artificially inoculated silt loam.....	32	69	84
Sterilized uninoculated silt loam.....	30	55	75

TABLE 5.—*The relation of soil moisture to the development of near-wilt in pea*

Description of soil	Variety of pea	Series No.	Dry soil		Medium-moist soil		Wet soil	
			Plants used ¹	Near-wilt index	Plants used ¹	Near-wilt index	Plants used ¹	Near-wilt index
			Number		Number		Number	
Inoculated silt loam.	Wilt Resistant Perfection.....	1	19	64	13	68	27	
	Alaska.....	2	12	54	17	50	17	
	Early Kay.....	1	41	51	51	47	54	
	Kay.....	2	20	51	29	47	20	
	Early Kay.....	2	19/6	55	19/8	61	20/4	
	Kay.....	2	19/8	67	19/0		20/14	
Naturally infested sandy loam.	Wilt Resistant Perfection.....	1	31	54	37	51	14	
	Alaska.....	2	17	44	18	46	19	
	Early Kay.....	1	31	41	56	41	42	
	Kay.....	2	17	41	20	40	17	
	Early Kay.....	2	16	82	14	39	19	
	Kay.....	2	20	80	17	54	14/12	

¹ In those cases in which all plants did not wilt permanently before the close of the experiment a fraction is given of which the numerator is the total number of plants and the denominator is the number of plants that did wilt permanently; the index is based on the data from the latter group only.

It will be seen from the data that in the case of Wilt Resistant Perfection and Alaska there was little difference between the indices in dry and medium-moist soil, while those in the wet soil were consistently the lowest. In the naturally infested soil disease development was generally more rapid, but the same relation prevailed between the dry, medium, and wet levels. Whether the greater rate of disease advance in the naturally infested soil is due to the supplementary effect of other organisms is not known, but it is recalled that the same relation occurred between artificially inoculated and naturally infested soil in the soil-temperature studies.

When the resistant varieties, Kay and Early Kay, are considered the soil-moisture effects are not so clear. The fact that disease development was so slow that usually only a small portion of the plants entered

into the index may account in part for the lack of conformity. In the naturally infested soil, where practically all of the plants wilted, the index of both resistant varieties was distinctly lower in the medium-moist soil than in either the dry or the wet soil.

DISCUSSION

Although the wilt and near-wilt diseases have closely related causal organisms and certain symptoms in common, they nevertheless are distinct in several respects. Perhaps the greatest important difference is that pea plants and varieties fall into two discontinuous groups insofar as resistance and susceptibility to wilt are concerned, while in the case of near-wilt, the distinction between susceptible and resistant types is not so clear. Under very favorable conditions for near-wilt all plants of resistant forms slowly but eventually succumb. The effect of the host plant itself upon the development of the disease is more striking in near-wilt. This is shown in the correlation between rate of disease development and the rate of blooming of the host. It is again shown in the increase in incubation period in proportion to host resistance. These varietal differences usually held at various soil temperatures and moistures.

The relative slowness of near-wilt development as compared with wilt development, regardless of variety, is outstanding. It is probably as important a factor as any in determining the fact that although the near-wilt organism is more widespread it is generally less destructive than wilt.

The fact that the optimum soil temperature for near-wilt is somewhat higher than that for wilt is not likely to be very important since near-wilt is nearly as destructive at 20° and 28° C. as at 24° and is limited at 16° to about the same degree as wilt. While susceptible varieties succumb most rapidly in wet soils the progress in moderately moist and dry soils is sufficiently rapid to indicate relatively little retardation in dry seasons.

Although the effects of temperature and moisture were quite consistent and clear-cut when susceptible varieties were used, it is important to note that they did not always coincide with those secured with resistant forms. These discrepancies warrant further study on these and other resistant varieties and need to be considered in the evaluation of plants in improvement of the pea for resistance to near-wilt.

SUMMARY

The investigations comprise a study of the temperature and soil-moisture relations of the near-wilt fungus (*Fusarium oxysporum* Schlecht. f. 8 Snyder) in relation to the pea plant.

On potato-dextrose agar the most rapid radial expansion of the organism occurred at 28° C. and the upper and lower limits for growth were somewhat above 36° and below 8°.

The near-wilt disease develops more slowly in a favorable environment than does wilt (*F. orthoceras* App. and Wr. var. *pisi* Linford).

Varieties differ in the rate at which near-wilt develops, the disease appearing more slowly as a rule in late-blossoming forms than in early-blossoming ones.

The optimum soil temperature for near-wilt is about 24° to 28° C. and it is thus about 5° higher than that for wilt. The disease develops readily, however, at temperatures as low as 20°; at 16° it is distinctly retarded. Air temperature has relatively little influence upon the disease.

In near-wilt-susceptible varieties there is little difference in the rate of wilting in dry and medium-moist soil, but it is consistently more rapid in moist soil. In the resistant varieties used the wilting is most rapid in medium-moist soil. The rate in all soils is sufficiently rapid, however, to indicate little retardation of the disease in dry seasons.

PHYSIOLOGICAL STUDIES OF LEMONS IN STORAGE¹

By ERSTON V. MILLER, *physiologist*, and HAROLD A. SCHOMER, *assistant physiologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*²

INTRODUCTION

It has long been known that the practice of storing lemons (*Citrus limonia* Osbeck) at a temperature of 40° F. and lower is conducive to the development of physiological disorders such as membranous stain and pitting. However, since higher temperatures are undesirable because they stimulate the growth of fungus parasites, many attempts have been made to treat the fruit in such a manner that the lower temperatures may be employed. A study of the metabolism of these fruits when subjected to various storage temperatures should yield information regarding fundamental causes of the physiological disorders.

Brooks and McColloch³ reported that pitting of lemons did not occur in storage at 60° F. and was seldom serious at 50° but was the chief limiting factor at lower temperatures if the storage period was extended beyond 4 weeks. They found that pitting was much worse at 32° and 36° than at 40°. Membranous stain of lemons occurred at 32°, 36°, 40°, 50°, and 60° in the following relative degree of severity, 1, 12, 22, 8, and 2, respectively, with usually only slight traces at 32° but with 75 to 100 percent of the fruits affected at 40°. These investigators were able to reduce the percentage of both disorders by a prestorage treatment with carbon dioxide or by waxing the fruit.

MATERIAL AND METHODS

Green California lemons were shipped under ventilation in carlot shipments from Los Angeles, Calif., to New York City and were forwarded to Washington, D. C., by ordinary express. Temperatures of 32°, 36°, 40°, 50°, and 60° F. were maintained in the cold-storage rooms at Arlington Experiment Farm, Arlington, Va. Enough lemons were stored at each temperature to permit each sample to consist of 30 fruits.

For chemical analyses the whole peel (flavedo and albedo) was separated from the pulp. Seeds were removed from the segments of pulp. For sugar and acid determinations, both peel and pulp were ground in a food chopper. Details of these analyses were identical with those described in a previous article.⁴ Glycoside content of the peel was determined by both Harvey's method⁵ and that of Darwin

¹ Received for publication February 18, 1939.

² The writers are indebted to Charles Brooks and L. P. McColloch, of this Division, for making samples available for chemical analysis and for supplying the pathological data.

³ BROOKS, CHARLES, and MCCOLLOCH, LACY P. SOME EFFECTS OF STORAGE CONDITIONS ON CERTAIN DISEASES OF LEMONS. *Jour. Agr. Research* 55: 795-809, illus. 1937.

⁴ MILLER, ERSTON V., and DOWD, OSCAR J. EFFECT OF CARBON DIOXIDE ON THE CARBOHYDRATES AND ACIDITY OF FRUITS AND VEGETABLES IN STORAGE. *Jour. Agr. Research* 53: 1-17, illus. 1936.

⁵ HARVEY, E. M. PHLORIDZIN. I. THE SIGNIFICANCE OF PHLORIDZIN IN APPLE AND PEAR TISSUE. II. THE HYDROLYSIS AND ESTIMATION OF PHLORIDZIN. *Oreg. Agr. Expt. Sta. Bull.* 215, 23 pp. 1925.

and Acton.⁶ The latter method proved more satisfactory, and only the results of this method are presented.

Reductase of the peel was determined as follows: 25 gm. of the ground peel was macerated by means of sand and a mortar and pestle and extracted with 100 ml. of water for 15 minutes. One milliliter of this filtered extract was added to 3 ml. of potassium permanganate (KMnO_4) (1.33 gm. per liter) and 2 ml. of N/10 oxalic acid. These solutions were held at 68° F. until they attained an amber color, and the time in minutes required to reach this end point was recorded.

For acetaldehyde determinations the pulp was not ground as for the sugar analysis. The details of the method have already been published.⁷ Acetaldehyde determinations on citrus peel were vitiated by the presence of interfering substances and were not considered reliable.

RESULTS

SUGAR

In tables 1 and 2 will be found the values for the sugar content of the lemon peel before, during, and after the storage period. With but one exception the results show a decrease in reducing sugar during storage for all temperatures. Usually the higher the storage temperature the greater the loss of reducing sugar. In the first experiment for 1934 (table 1) the peel of the fruits at 50° F. lost 23 percent of its reducing sugar in 13 weeks; in the second experiment of the same year the peel of the fruits at 50° lost 33 percent of its original reducing sugar in 11 weeks. The 32° lots in the first experiment showed a slight gain, while those in the second experiment had lost 5.7 percent at the end of the storage period. In 1935 (table 2) the peel of the fruit stored at 50° lost 26.5 percent of its original reducing sugar in 15 weeks; the 32° lot lost 15.7 percent of its reducing sugar during the same time. For some unknown reason the lots at 36° and 40° in 1935 lost more sugar than other lots. These losses in reducing sugar are also reflected in the total-sugar values. Sucrose occurs in relatively small quantities in both the peel and pulp of lemons, and it is difficult to interpret the values for this substance; it sometimes increased and sometimes decreased, but with no apparent consistency.

In 1935 the pulp, as well as the peel, was analyzed for sugar (table 2). The results for the pulp were quite similar to those for the peel. Reducing sugar and total sugar diminished in quantity during the storage period, and the decrease was greatest at the higher temperatures. There was still less sucrose in the pulp than in the peel.

In all these results there is nothing to indicate that the changes in sugar content might be related to pitting of the peel. The greater loss of reducing sugar at 36° and 40° F. in the 1935 experiments suggests a relationship with pitting, but these results had not been observed in the 40° lots in 1934 and can hardly be considered as significant.

⁶ DARWIN, FRANCIS, and ACTON, E. HAMILTON. PRACTICAL PHYSIOLOGY OF PLANTS. Ed. 3, 340 pp., illus. Cambridge. 1901. (See p. 285.)

⁷ MILLER, ERSTON V. DISTRIBUTION OF ACETALDEHYDE AND ALCOHOL IN THE APPLE FRUIT. Jour. Agr. Research 53: 49-55. 1936.

TABLE 1.—*Sugar content*¹ of peel of lemons stored at three temperatures, 1934

MARCH TO JUNE (EXPERIMENT 1)

Storage temperature (° F.)	Reducing sugar			Sucrose			Total sugar		
	At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—	
		7 weeks	13 weeks		7 weeks	13 weeks		7 weeks	13 weeks
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
50.....	4.77	3.93	3.65	1.09	0.84	0.64	5.86	4.77	4.29
40.....	4.77	4.29	4.11	1.09	1.05	1.12	5.86	5.34	5.23
32.....	4.77	5.05	5.09	1.09	1.10	1.57	5.86	6.15	6.66

JUNE TO SEPTEMBER (EXPERIMENT 2)

		4 weeks	11 weeks		4 weeks	11 weeks		4 weeks	11 weeks
50.....	4.01	3.35	2.68	0.61	0.70	0.72	4.62	4.05	3.40
40.....	4.01	3.80	2.76	.61	.89	.79	4.62	4.69	3.55
32.....	4.01	3.74	3.78	.61	.82	.80	4.62	4.56	4.58

¹ Percentage of fresh weight.TABLE 2.—*Sugar content*¹ of peel and pulp of lemons stored at various temperatures, January to May 1935

Storage temperature (° F.)	Peel									Pulp								
	Reducing sugar			Sucrose			Total sugar			Reducing sugar			Sucrose			Total sugar		
	At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—		At beginning of storage period	After storage for—	
		9 weeks	15 weeks		9 weeks	15 weeks		9 weeks	15 weeks		9 weeks	15 weeks		9 weeks	15 weeks		9 weeks	15 weeks
	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>	<i>Pct.</i>
60.....	4.15	3.58	2.98	0.58	0.64	0.71	4.73	4.22	3.69	1.92	1.53	1.32	0.09	0.26	0.10	2.01	1.79	1.42
50.....	4.15	3.99	3.05	.58	.64	.52	4.73	4.63	3.57	1.92	1.91	1.43	.09	.21	.36	2.01	2.12	1.79
40.....	4.15	3.28	2.86	.58	.74	.66	4.73	4.02	3.52	1.92	1.41	1.49	.09	.34	.32	2.01	1.75	1.81
36.....	4.15	3.35	2.67	.58	.77	.53	4.73	4.12	3.20	1.92	1.60	1.70	.09	.43	.12	2.01	2.03	1.82
32.....	4.15	3.78	3.50	.58	.79	.77	4.73	4.57	4.27	1.92	1.63	1.67	.09	.38	.24	2.01	2.01	1.91

¹ Percentage of fresh weight.

GLYCOSIDES AND TOTAL ACIDITY

The glycoside content of the peel is shown in table 3. These substances are present in relatively small amounts in fruits. The glycoside content of the peel at the end of 4 weeks' storage differed little from the original amount. The fluctuations at 40° and 50° F. are doubtless due to sampling errors. At 32° the quantity increased from 0.74 to 0.90 percent in 4 weeks. On the other hand, the 11 weeks' sample showed definite increases in glycosides. From the original sample, which had a glycoside content of 0.74 percent, the lots stored at 50°, 40°, and 32° showed an increase to 1.12, 1.26, and 1.08 percent, respectively.

The results for total acidity of the peel were somewhat similar to those for glycosides (table 3). The total acidity at the end of 7 weeks' storage showed no increase in the 40° and 50° F. lots and only a slight increase at 32°. At the end of 13 weeks there was a consistent increase in total acidity of the peel. The original sample at the beginning of storage contained 0.16 percent of acid. At the end of the storage period the 50°, 40°, and 32° lots contained 0.23, 0.18, and 0.21 percent of total acid, respectively. There was no apparent relation between total acidity and pitting or between glycosides and pitting.

TABLE 3.—*Glycoside content*¹ and *total acidity*² of peel of lemons stored at three temperatures

Storage temperature (°F.)	Glycosides			Total acidity		
	At begin- ning of storage period	After storage for—		At begin- ning of storage period	After storage for—	
		4 weeks	11 weeks		7 weeks	13 weeks
50.....	<i>Percent</i> 0.74	<i>Percent</i> 0.66	<i>Percent</i> 1.12	<i>Percent</i> 0.16	<i>Percent</i> 0.14	<i>Percent</i> 0.23
40.....	.74	.70	1.26	.16	.15	.18
32.....	.74	.90	1.08	.16	.18	.21

¹ Percentage of fresh weight.

² Percentage of acid as citric in fresh peel.

ACETALDEHYDE

The acetaldehyde content of the lemon flesh at various storage periods is presented in table 4. Inasmuch as the quantity evolved from the same variety of fruit may vary with different pickings and with different years, the results of all four experiments are included in the table. Acetaldehyde was highest in the last samples taken, if the storage period extended to 13 or 15 weeks, because many of the fruits had been held until internal break-down had begun. The amount of acetaldehyde at this period ranged from 0 to 1.3 mg. per 100 gm. of fresh material. A slight amount had accumulated in the midstorage sample. In several instances there was slightly more acetaldehyde in the 40° F. lots than in others, but this was not consistent. In fact, there was nothing to indicate that physiological disorders, like pitting and membranous stain, could be traceable to accumulation of acetaldehyde at these lower temperatures.

TABLE 4.—Acetaldehyde content¹ of pulp of lemons stored at various temperatures, 1934 and 1935

1934			
Storage temperature (°F.)	Acetaldehyde		
	At beginning of storage period	After storage for—	
		7 weeks	13 weeks
	Milligrams	Milligrams	Milligrams
50.....	0.0	0.7	0.9
40.....	0	.7	1.0
32.....	0	.9	0
1935 (EXPERIMENT 1)			
		9 weeks	15 weeks
60.....	0.0	0.2	0.0
50.....	0	.2	.2
40.....	0	.3	.6
36.....	0	.4	1.3
32.....	0	.1	.9
1935 (EXPERIMENT 2)			
		6 weeks	8 weeks
60.....	0.0	0.0	0.0
50.....	0	0	0
40.....	0	.1	.2
36.....	0	0	.3
32.....	0	0	.3

¹ Milligrams per 100 gm. of fresh pulp.

REDUCTASE

The results for reductase determinations are presented in table 5. Reductase is expressed as time in minutes required for an aqueous extract of the peel to reduce a standard potassium permanganate solution. The reductase value for the original or prestorage samples ranged from 3.00 to 9.67 minutes. Subsequent studies indicated that this variation may be due to difference in maturity of fruit. The reductase values are therefore relative and must be compared only with lots of fruit in the same experiment. In all four of the experiments reported, the time required for reduction of the potassium permanganate was greatest for the fruits stored at temperatures of 40° F. or lower. Thus, in the midstorage samples of the first experiments for 1934 the value for the 40° lot exceeds that for the 50° lot by 1.83 minutes, and that for the 32° lot by 1.15 minutes. In the second experiment the excess of reducing time of the midstorage 40° lot over the 50° lot was 7.22 minutes, and over the 32° lot it was 4.15 minutes.

TABLE 5.—*Reductase activity*¹ of peel of lemons stored at various temperatures, 1934 and 1935

1934 (EXPERIMENT 1)					
Storage temperature (°F.)	Reductase activity			Disease record at end of storage	
	At beginning of storage period	After storage for—		Area affected by pitting	Fruit showing membranous stain
		7 weeks	13 weeks		
	Minutes	Minutes	Minutes	Percent	Percent
50.....	3.00	4.12	3.79	-----	31.4
40.....	3.00	5.95	5.16	-----	99.9
32.....	3.00	4.80	4.74	-----	6.6
1934 (EXPERIMENT 2)					
		4 weeks	11 weeks		
50.....	6.78	6.86	5.50	-----	88.2
40.....	6.78	14.08	5.97	-----	91.3
32.....	6.78	9.93	7.20	-----	40.0
1935 (EXPERIMENT 1)					
		9 weeks	15 weeks		
60.....	9.67	5.00	3.27	29.3	19.4
50.....	9.67	4.33	4.55	11.7	73.3
40.....	9.67	7.33	8.75	40.0	93.3
36.....	9.67	8.29	9.25	76.5	83.8
32.....	9.67	9.95	8.52	60.0	6.7
1935 (EXPERIMENT 2)					
		6 weeks	8 weeks		
60.....	7.66	6.37	6.25	0	20.0
50.....	7.66	6.37	5.75	0	83.3
40.....	7.66	6.66	7.33	12.6	96.3
36.....	7.66	8.91	7.33	22.3	30.9
32.....	7.66	7.65	7.87	5.7	6.9

¹ Expressed as time in minutes required by aqueous extract of peel to reduce potassium permanganate.

In the 1935 experiment (table 5) a similar relationship held. It may be shown in a different manner. In the midstorage sample of the first experiment the average value for the temperatures 50° and 60° F. was 4.67, while that for the lower temperatures (40°, 36°, and 32°) was 8.52, almost twice as high. In the second experiment of the same year the average value for the temperatures 60° and 50° was 6.37 and that for the lower temperatures was 7.74. Since reductase activity is indicated as the time required to reduce the potassium permanganate solution, a high value in the table indicates a low reductase value. Therefore, the lots stored at temperatures that produced the most pitting (40°, 36°, and 32°) showed the lowest reductase activity. While there is no direct correlation between these values and pitting in lemons, the temperatures that produced the most pitting in storage (40°, 36°, and 32°) showed the lowest reductase activity.

DISCUSSION

The sugar analyses made of the peel of lemons during storage do not suggest any abnormality in the metabolism of the fruit at any tem-

perature studied. One would expect the reducing sugar to be consumed during the respiratory processes. The greater loss at higher temperatures is also natural. Similarly, the acetaldehyde determinations do not indicate any abnormality in the fruit. In these experiments the fruits yielded varying amounts of acetaldehyde after they had been in storage awhile. No unusual quantities were found in fruit stored at 36° or 40° F., the temperatures so conducive to membranous stain in lemons. Whatever the factors that cause these physiological disorders may be, they apparently do not affect sugar consumption or the formation of acetaldehyde.

No additional information on physiological disturbance was obtained from the acid and glycoside values. The slight increase in total acid as storage was continued may be the result of dehydration. The glycoside content of the peel shows a much greater increase. This may be a natural aging process. Again, there is nothing in these results to indicate that the fruit at the lower temperatures will show a higher percentage of physiological disorders.

A slightly different result is obtained from the reductase studies. The reductase activity in the peel of the fruit stored at 32°, 36°, and 40° F. was always lower than in that stored at 50° and 60°. The first three temperatures are known to be most conducive to the production of pitting in lemons. Apparently some substance in the peel is oxidized more rapidly at these lower temperatures and is therefore not oxidized by the potassium permanganate solutions. The fact that sometimes the pits assume a dark appearance also suggests the action of oxidases. Reducing sugar and acetaldehyde might account for some part in the reduction of potassium permanganate, but the reaction is certainly enzymatic, because boiling the peel destroys its ability to reduce potassium permanganate at different rates.

SUMMARY

Biochemical studies were made of lemons stored at 32°, 36°, 40°, 50°, and 60° F.

The peel was analyzed for sugar, glycosides, acids, and reductase activity before, during, and after storage. The flesh was analyzed for sugar and acetaldehyde.

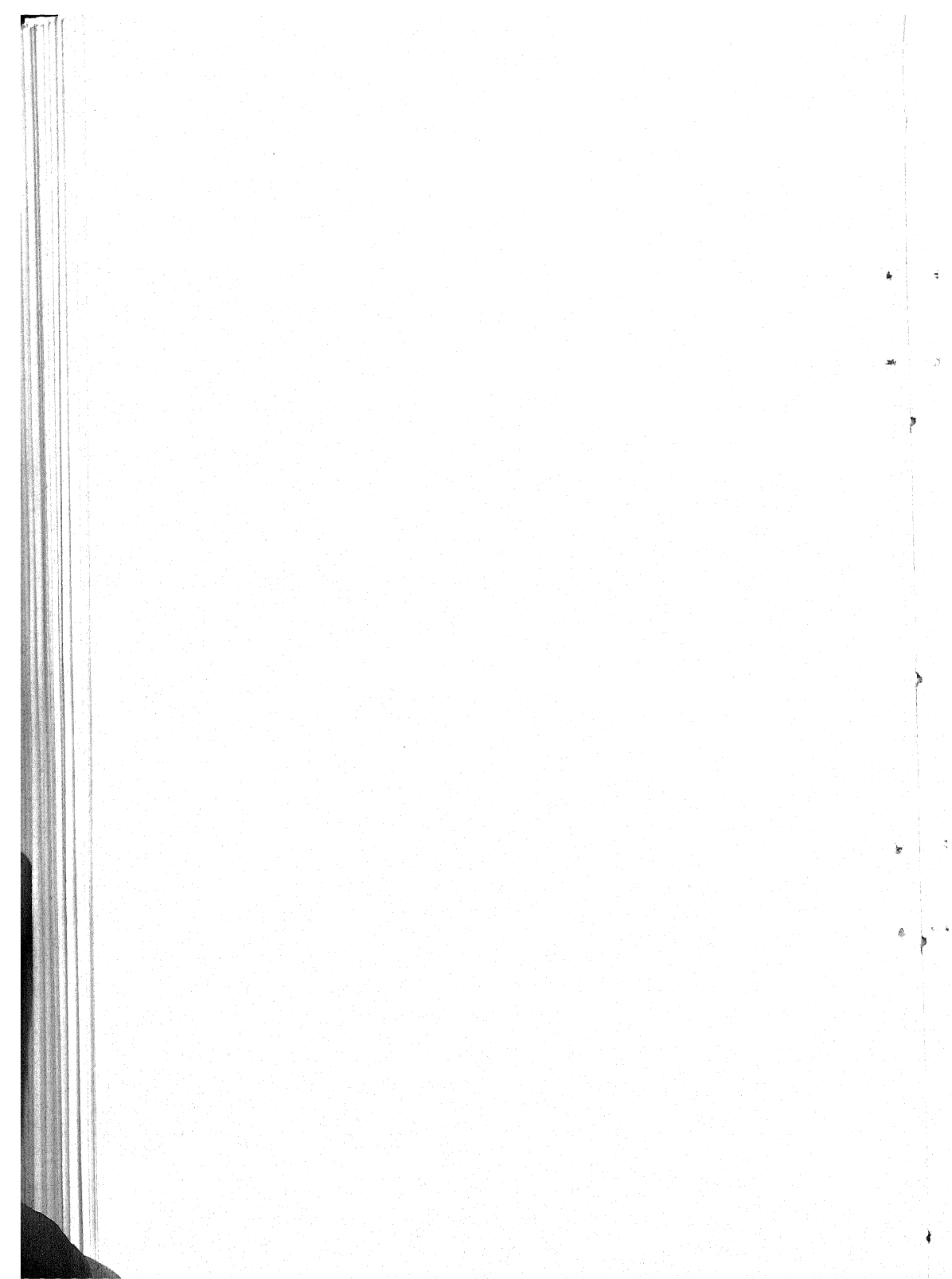
Reducing sugar and total sugar in both peel and flesh diminished in quantity during storage of the fruit. These tissues contained only slight amounts of sucrose.

Both total acids and glycosides in the peel increased during storage.

Varying amounts of acetaldehyde were found in the flesh at the time of the midstorage and final sampling dates.

No relation was found between any of the above-mentioned substances and the development of pitting and membranous stain in the fruit.

Reductase activity of the peel, as measured by the rate of reduction of potassium permanganate solutions, was consistently lower for the samples stored at 32°, 36°, and 40° F. than for those stored at 50° and 60°. Inasmuch as the lower temperatures (32°, 36°, 40°) are most conducive to development of pitting of lemons in storage, it is suggested that oxidizing enzymes may play a role in the development of this disorder.



INFECTION AND REINFECTION EXPERIMENTS WITH BANG'S DISEASE¹

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INTRODUCTION

The experiments reported here are concerned with: (1) The effect of time of breeding and stage of gestation on the agglutinin response of animals exposed to *Brucella abortus*; (2) the channels of entrance of the bacteria into the animal body; and (3) the result of reinfection of animals.

The course of Bang's disease in female cattle following natural exposure is generally recognized as being extremely complicated. The results that follow infection depend to a large extent on the status of the animal at the time infection occurs. The term "status of the animal" as used in this paper refers to such variables as age, puberty, the presence or absence of pregnancy, and the duration of gestation.

The first point of interest in these experiments is associated with the variable results obtained with six groups of cattle having different breeding histories at the time of their experimental infection. Observations similar to some of these have been recorded by other investigators; however, the relationship of these results to the status of the animal at the time of infection appears not to have received particular emphasis.

The second phase of these experiments pertains to the channels of entrance of *Brucella abortus* organisms into the animal body. Numerous investigations have shown that there are various avenues through which the bacteria may gain entrance to the animal body; namely, the digestive tract, the conjunctival membrane, the teat canal, the vagina, the unbroken skin, and by subcutaneous or intravenous injection.

There still remains some question as to whether or not the channel of entrance has an effect on the reaction of the animal following infection. In other words, do the reactions of particular tissues influence the course of the disease?

In these experiments some infections were per vagina, some per conjunctiva, and some per os.

In the vaginal exposures the vagina may or may not have been the sole channel of entrance of the infection (as will be discussed later), nevertheless the results warrant consideration.

The third phase of the work relates to reinfection and is one concerning which data are relatively meager. Vaccination experiments, in which animals have been inoculated subcutaneously with organisms of various degrees of pathogenicity, have frequently included reinfections, but the data usually have not been analyzed in terms of reinfection experiments.

There is considerable clinical evidence suggesting that many of the symptoms attending this disease are associated with repeated rein-

¹ Received for publication April 19, 1939.

fections of the animal rather than with a single, initial infection. However, so far as the writers know, but few controlled experiments have been undertaken to verify such clinical observations. The experiments reported here appear to have a bearing on this phase of the course of Bang's disease under natural conditions.

EXPERIMENTAL PROCEDURE

Infections were produced in the following ways: (1) By introducing *Brucella abortus* organisms into the vagina; (2) by instilling the organisms into the conjunctival sac; and (3) by feeding grain mixed with suspensions of the bacteria. Several different strains of *Br. abortus* isolated from cattle were grown for 48 to 96 hours on the usual pork- or liver-agar media. The growths were washed from the media with physiologic salt solution and the bacterial suspensions standardized to 10 times tube No. 2 of the McFarland nephelometer. Vaginal exposures were made by slowly injecting 20 to 40 cc. of the fresh bacterial suspensions into the vagina, eye exposures by instillation of the suspension into the lachrymal sac, and oral exposure by the daily feeding on 3 successive days of a culture from one tube of medium mixed with the grain for each cow. Eighteen of the cattle inoculated through the vagina in the earlier tests were kept muzzled, except at feeding and watering time, for from 6 to 8 weeks after inoculation. This was done to minimize the chances of the bacteria gaining entrance to the alimentary canal. During the time that the animals were muzzled they were housed constantly in separate stanchions with rails between each animal. The muzzling was discontinued in later experiments, after it seemed apparent that animals regularly became infected by this method of exposure. (See discussion of group 1.)

Agglutination blood titers were determined at approximately 1-month intervals beginning at the time of exposure of the animal. Antigen for the tests was prepared essentially in accordance with the method recommended by the United States Live Stock Sanitary Association.²

All the animals were free from Bang's disease at the time they were placed in the experiments, as judged by clinical histories and repeated negative agglutination tests. Six different groups of cattle were used in the experiment, viz: (1) Heifers exposed to *Brucella abortus* during difference stages of pregnancy; (2) heifers exposed prior to pregnancy; (3) heifers exposed prior to breeding and reexposed subsequent to pregnancy; (4) heifers exposed prior to and reexposed subsequent to breeding that did not result in pregnancy; (5) heifers exposed before their first pregnancy and reexposed during their second pregnancy; and (6) cows exposed prior to and subsequent to pregnancy.

EXPERIMENTAL DATA

GROUP 1: HEIFERS EXPOSED DURING DIFFERENT STAGES OF PREGNANCY

Twelve heifers of breeding age were included in the first group. Vaginal inoculations were administered at different stages of gestation, ranging from approximately 1½ to 5 months after conception occurred. The data are given in table 1.

² FITCH, C. P., chairman. REPORT OF COMMITTEE ON BANG'S DISEASE. Amer. Vet. Med. Assoc. Jour (n. s. 35) 82: 335-344. 1933.

TABLE 1.—Results with heifers given vaginal injections of *Brucella abortus* at different stages of pregnancy

Heifer No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer (1: figure shown) ¹											
				Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
15	Nov. 23	Jan. 14	Aborted July 15.	—	—	—	0	400	1,600	200	800	800	6,400	—	—
17	Nov. 25	do	Aborted Apr. 25.	—	—	—	0	200	800	400	1,600	—	—	—	—
18	Dec. 4	do	Aborted June 23.	—	—	—	0	200	3,200	200	3,200	1,600	25,600	—	—
7	June, pasture.	Oct. 5	Aborted Jan. 17.	0	1,600	1,600	1,600	—	—	—	—	—	—	—	—
8	do	do	do	0	1,600	1,600	1,600	—	—	—	—	—	—	—	—
12	do	do	Aborted Jan. 13.	0	1,600	800	400	—	—	—	—	—	—	—	—
66	Jan. 27	Mar. 26, June 20, (eye).	Calved Nov. 11.	—	—	—	—	—	0	100	100	50	50	25	50
81	Dec. 6	Mar. 26	Aborted May 27.	—	—	—	—	—	0	1,600	3,200	6,400	3,200	1,600	6,400
82	Dec. 5	do	Aborted June 9.	—	—	—	—	—	0	1,600	6,400	6,400	6,400	6,400	6,400
24	June 17	Oct. 23	Aborted Dec. 13.	0	800	400	1,600	3,200	3,200	100	—	—	—	—	—
32	June 6	do	Aborted Jan. 21.	0	400	200	1,600	3,200	800	400	—	—	—	—	—
34	May 20	do	Aborted Jan. 31.	0	400	200	6,400	3,200	3,200	3,200	—	—	—	—	—

¹ 0 = negative at 1:25. The numerals 25, 50, 100, etc. = complete at 1:25, 1:50, 1:100, etc.

Results.—All the heifers except one (No. 66) developed relatively high agglutination titers (1:200 dilution or above) within approximately 1 month after inoculation. Such agglutination titers persisted as long as the animals were continued in the experimental herd. Each of these 11 heifers aborted. The abortions occurred at various times between the fifth and eighth month of gestation. Heifer No. 66 developed a positive agglutination reaction (1:100 dilution) after approximately 1 month, but within 60 days the titer had fallen to the 1:50 dilution (suspicious reaction). A second inoculation, this time through the conjunctiva, was administered to this animal approximately 3 months after her first exposure. No alteration of the existing suspicious agglutination reaction was observed as a result of this second exposure. This heifer went through a normal gestation and gave birth to a vigorous living calf.

Discussion.—These results strongly suggest that heifers can be infected readily by vaginal inoculation of relatively large doses of virulent *Brucella abortus* organisms. It is important to recognize that under the existing conditions of experimental exposure all possibilities of bacteria entering the animal body through channels other than the vagina were not eliminated. It is conceivable that some organisms may have escaped from the vagina and in some manner have come in contact with other tissues such as the conjunctiva or skin. While such chance transmission of bacteria is possible it seems unlikely that it would have occurred regularly in all of the animals that were muzzled after vaginal inoculations. It seems more reasonable to assume that the infection was the direct result of absorption of the bacteria through the vaginal mucosa.

The results further confirm the generally accepted opinion that the noninfected pregnant heifer usually is highly susceptible to *Brucella*

infections and that the incidence of abortions in infected cattle of this class is high.

GROUP 2: HEIFERS EXPOSED PRIOR TO PREGNANCY

Four heifers of breeding age were included in the second group. Vaginal inoculations were administered at periods ranging from 56 to 75 days prior to breeding. The data are given in table 2.

TABLE 2.—*Agglutinin titers of heifers given vaginal injections of Brucella abortus prior to breeding*

Heifer No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer ¹ (1 : figure shown)					
				Jan.	Feb.	Mar.	Apr.	May	June
13	Mar. 11	Jan. 14	Calved Dec. 16.....	0	400	100	200	25	0
14	Mar. 25do....	Calved Dec. 8.....	0	50	50	25	0	0
20	Mar. 8do....	Calved Dec. 12.....	0	100	400	0	0	200
23	Mar. 30do....	Calved Jan. 13.....	0	200	3, 200	400	200	200

See footnote 1, table 1.

Results.—There was no uniformity in the agglutinin development of these animals except that all showed some degree of response, as will be observed in the accompanying data (table 2). The titers were not high consistently and did not persist over a long period. All the heifers had normal gestation periods and gave birth to vigorous, living calves.

Discussion.—The results obtained with this group show a distinct difference in the effects of *Brucella abortus* infection acquired before pregnancy as compared with those observed in the pregnant heifers of group 1.

When infection occurs in heifers at least 60 days before pregnancy the animals usually do not abort. This is consistent with the results frequently reported for heifers inoculated subcutaneously with virulent organisms prior to breeding.

The results further suggest that vaginal inoculations caused sufficient tissue reaction in these heifers to protect them from abortion in the first pregnancy. In other words, these results suggest that it is the status of the animal at the time of infection rather than the channel of exposure that largely determines the results of the infection.

GROUP 3: HEIFERS EXPOSED PRIOR TO BREEDING AND REEXPOSED DURING PREGNANCY

The third group consisted of three heifers approximately 14 months of age. They were therefore beyond the age of puberty but had not reached breeding age at the time of the first exposure. Two of the heifers were exposed through the vagina, the third by way of the conjunctiva, 4, 5, and 7 months, respectively, prior to breeding.

After conception but before the termination of the gestation period, each heifer received two additional exposures to *Brucella abortus*. These subsequent exposures were per conjunctiva except one, which was per vagina. The data, including time intervals and the stage of

pregnancy in each case at the time of these subsequent exposures, are given in table 3.

TABLE 3.—*Heifers exposed to Brucella abortus prior to breeding and reexposed during pregnancy*

Heifer No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer ¹ (1: figure shown)											
				Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
67	Feb. 7	Aug. 18, eye.....	Calved Nov. 24.	0	1,600	200	100	100	100	50	50	100	100	100	50
		Apr. 26, eye.....													
		June 20, eye.....													
77	Dec. 6	Aug. 18, vaginal.....	Calved Sept. 8	0	1,600	50	0	0	25	0	0	25	25	100	100
		Mar. 26, eye.....													
		June 20, eye.....													
80	Jan. 3	Aug. 18, vaginal.....	Calved Oct. 3	0	1,600	50	25	25	25	0	0	50	100	100	50
		Mar. 26, vaginal.....													
		June 20, eye.....													

¹ See footnote 1, table 1.

Results.—All three heifers developed relatively high agglutination titers (1:1,600 dilution) within approximately 1 month after exposure. In all three the maximum titers dropped sharply after 1 month. The reinfection exposures to *Brucella abortus* were followed by only relatively slight elevations of the agglutinin titers in some instances and none or decreased titers in others.

All three animals had normal gestations and gave birth to vigorous living calves.

Discussion.—The most interesting feature of this group is the failure of reexposures to appreciably and consistently alter the agglutinin titers or to interfere with normal gestation. It appears that these three heifers acquired a sufficient tissue reaction as a result of exposure prior to breeding to exclude any appreciable effects from subsequent reexposures during the first pregnancy.

GROUP 4: HEIFERS EXPOSED PRIOR TO BREEDING AND REEXPOSED SUBSEQUENT TO BREEDING THAT DID NOT RESULT IN PREGNANCY

The fourth group consisted of four heifers approximately 14 months of age. As in the case of group 3 these heifers were beyond the age of puberty, but were not of breeding age at the time of the initial exposure.

Three of the heifers were exposed through the vagina, one 6 months and the others 7 months before breeding. The fourth was exposed through the conjunctiva, 6 months before breeding. Exposures made subsequent to breeding were administered once in one animal and twice in each of the other three. The intervals and channels of re-exposure along with agglutination results are given in table 4.

TABLE 5.—*Heifers exposed to Brucella abortus prior to first pregnancy and reexposed during second pregnancy*

Heifer No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer (1: figure shown) ^{1 2}											
				Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
13	Mar. 11	Jan. 14, vaginal....	Calved Dec. 16	0	400	100	200	25	0	0	0	0	..	0	25
	June 6	Oct. 23, 24, 25, oral; Oct. 29, vaginal.	Calved Mar. 22												
20	Mar. 8	Jan. 14, vaginal....	Calved Dec. 22	0	100	100	400	0	0	200	50	25	25	100	100
	May 7	Oct. 23, 24, 25, oral;	Calved Feb. 6												
14	Mar. 25	Jan. 14, vaginal....	Calved Jan. 8	0	50	50	25	0	..	0	0	0	..	0	25
	May 1	Oct. 23, 24, 25, oral.	Calved Feb. 13												
23	Mar. 30	Jan. 14, vaginal....	Calved Jan. 13	0	200	1,600	200	200	200	100	50	200	400	200	200
	May 9	Oct. 23, 24, 25, oral. Oct. 29, vaginal....	Calved Jan. 16 Calf weak at birth, survived												

¹ See footnote 7, table 1.² No data for February to August, inclusive, of the second year.

Results.—Three of the heifers developed positive agglutination reactions (1:100 dilution or above) within 30 days after the initial vaginal inoculations. These titers rather rapidly receded to low positive, suspicious, and even negative zones and fluctuated somewhat during the first pregnancy. The fourth heifer showed a reaction in only 1:50 dilution for a brief period and then returned to a negative reaction, which persisted throughout the first pregnancy. All four heifers had normal gestation periods and gave birth to vigorous living calves from their first pregnancies.

During the second pregnancy the agglutination titer showed no appreciable consistent alteration following the reinfection exposures. Three of the heifers had normal gestation periods in their second pregnancies and gave birth to vigorous living calves. Heifer No. 23 gave birth to a live calf on the two hundred and fifty-second day of gestation. The calf was weak at birth but survived.

Discussion.—The results obtained with this group should be compared with those of group 3. The essential difference in the two tests is the reexposure of the animals. Initial exposures of each group were made prior to the breeding for first pregnancy. Group 3 animals were reexposed during their first pregnancy, whereas those of group 5 were not reexposed until their second pregnancy.

The general trend of the agglutinin titers did not appear to be materially different in the two groups. The resistance to the act of abortion, presumably induced by the initial exposure of the animals, appeared to be somewhat less complete when reexposure was delayed until the time of second gestation.

TABLE 6.—Cows exposed to *Brucella abortus* prior to breeding and reexposed during second pregnancy¹

Cow No.	Date of breeding	Date of exposure	Outcome of pregnancy	Blood agglutinin titer 2:3 (1: figure shown)											
				Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July
2	Feb. 18	Aug. 18, eye	Calved Nov. 27	0	1,600	400	100	100	50	25	50	25	50	50	50
		Apr. 26, eye													
		June 20, vaginal													
		Aug. 18, vaginal													
6	Feb. 8	Aug. 26, vaginal	Calved Nov. 4	0	1,600	400	100	100	100	25	25	100	100	100	100
		June 20, eye													
		Aug. 18, vaginal													
		Mar. 26, vaginal													
35	Dec. 14	Aug. 18, vaginal	Calved Sept. 21	0	1,600	50	25	0	0	0	25	50	25	25	25
		Mar. 26, vaginal													
		June 20, eye													
		Aug. 18, eye													
3	Feb. 25	Apr. 26, vaginal	Calved Dec. 17	0	3,200	3,200	3,200	1,600	400	200	200	400	400	400	1,600
		June 20, eye													
		Aug. 18, vaginal													
		Mar. 26, eye													
7	Jan. 2	Aug. 18, vaginal	Calved Oct. 16	0	1,600	200	25	50	50	0	0	200	100	50	50
		June 20, eye													
		Aug. 18, vaginal													
		Apr. 26, eye													
64	Mar. 12	Aug. 18, eye	Calved Dec. 19	0	1,600	-----	200	200	200	50	50	25	100	200	100
		June 20, eye													
		Aug. 18, eye													
		Apr. 26, eye													

¹ Animals were pasture-bred for first pregnancy; breeding dates unknown.² See footnote 1, table 1.³ No data for January of the second year.

GROUP 6: COWS EXPOSED PRIOR TO BREEDING AND REEXPOSED DURING THE SECOND PREGNANCY

Six young cows, each having calved once previously, were included in a sixth group. Virulent *Brucella abortus* organisms were administered per vagina to four of these cows and per conjunctiva to the other two. The animals were not bred until 4 to 7 months after the first experimental exposure. Each received two reinfection exposures during their second pregnancy. Reinfection exposures were administered to the cows during the first half of their gestation periods. Some of the reinfection exposures were per vagina and others per conjunctiva.

Results.—Each of the animals developed a high agglutination reaction within 30 days after the first exposure (table 6). These high titers receded materially within 30 to 90 days. In general the titers dropped to the suspicious or low positive zones (1:25 to 1:100) except for one animal (No. 3), which carried a sufficient titer to be classified as positive every time tests were conducted. The increase in agglutinin titer of the different cows following their first reinfection exposure varied somewhat, but was never so great as that following the initial exposure. The second reexposure did not regularly cause similar titer increases.

All the animals passed through normal gestation periods and gave birth to vigorous, living calves from their second pregnancy.

Discussion.—A comparison of the results obtained with these cows and those obtained with the heifers of group 3, shows no significant difference in agglutinin response attributable to the gestation previous to initial exposure.

The results indicate that these cows developed sufficient tissue reaction from their first exposure prior to their second gestation to protect them against the act of abortion even when subjected to two reinfection exposures during their second pregnancy.

DISCUSSION AND CONCLUSIONS

Many students of Bang's disease have observed that infected cows usually abort but once or twice and thereafter carry their calves full time. In other words, after infection the female bovine usually develops a resistance to the act of abortion after having had the disease for a sufficient length of time to abort one or two calves. It is generally conceded, as a result of clinical experience and infection and vaccination experiments, that some infected cows do not abort. The experiments herein reported confirm these observations. They strengthen the opinion that cows which become infected while they are in a nonpregnant state are not likely to abort their next calves and may never abort. Infection of the heifer or cow sometime prior to pregnancy appears to place her ahead of the animal that is pregnant at the time of infection in that she attains the stage of the disease in which she is less apt to abort without ever having passed through the stage at which animals usually do abort.

It is interesting to note that the results of these experiments are essentially the same as those obtained in vaccination experiments of a past era in which pathogenic cultures were administered subcutaneously to nonpregnant heifers and cows. The results tend to show that it is not the method of administration nor the channel of entrance of *Brucella abortus* organisms, but rather the status of the animal at the time of exposure, that determines what will happen insofar as the act of abortion is concerned.

It should be recognized that these experiments were designed only to study the agglutination response and the incidence of abortion when different classes of cattle were exposed to virulent *Brucella abortus* organisms. The study does not include the question of producing carrier spreaders of Bang's disease. It has long been known that abortion is but one of the symptoms of Bang's disease. If the problems concerned with this disease consisted only in preventing abortions, which is certainly not the case, its solution would be relatively simple since these and other experiments have shown that it is possible to control this symptom to a considerable degree by exposing animals at the proper time.

The results of these experiments strongly suggest that it is relatively easy to infect the female bovine experimentally by placing a suspension of virulent *Brucella abortus* organisms into the vagina. Furthermore, infections through this channel produce effects essentially the same as when infection enters the body through other avenues.

Under the conditions of these experiments, reinfection exposures did not appear to cause definite and consistent alterations in the agglutinin content of the blood of previously exposed animals, nor did they significantly interfere with the animals' capacity to carry their calves to maturity. This should not, however, be interpreted as conclusive evidence as to the role of reinfection in Bang's disease under conditions other than those included in these experiments. Intermittent or continuous reexposure occurring at various intervals before breeding might also affect the outcome of pregnancy.

GROWTH OF MILLET IN QUARTZ SAND AND IN SAND-SOIL MIXTURES¹

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INTRODUCTION

Data available from previous investigations show the comparative growth made by millet (*Setaria italica* (L.) Beauv.) in quartz sand and in many sand-soil mixtures. With the usual nutrient salts supplied in supposedly optimum amounts, millet in nearly every case yielded more in the sand-soil mixtures than in pure quartz sand. This paper reports the results of experiments that have been conducted to determine how the admixture of a little soil improves quartz sand as a medium for growing millet.

METHODS

Part of the data considered in this paper is taken from previously published experiments dealing with other subjects (2, 3, 4).³ The procedure followed in conducting the tests has, therefore, been described in detail and is only summarized here.

Glazed earthenware pots of 1-gallon capacity, holding about 5,000 gm. of sand, were used. The moisture content of the sand or sand-soil mixture, determined by weighing the pots, was maintained at 17 percent (15 percent in some cases) by adding distilled water. Ten millet plants per pot were grown for 23 to 42 days according to the time of year. Usually the heads were about to appear when the plants were cut. The weight varied markedly between plants grown in mid-winter and those grown in early summer.

A uniform mixture of soil or other insoluble material with the quartz sand was obtained by first moistening the sand with 1 percent of water to prevent segregation of materials. The fertilizer salts to be added to the pot were dissolved in the distilled water used to make the sand to moisture content. The fertilizer salts applied were the No. 2 mixture (see table 8), unless otherwise specified.

REVIEW OF LITERATURE

Apparently the effect of soil applications in increasing the yield of sand cultures has not been investigated extensively. Studies have appeared, however, from time to time, dealing with the increased yields produced by adding peat, clay, silica gel, and various nonnutritive solids to quartz sand (9).

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² Thanks are due E. H. Bailey, of the Division of Soil Survey, for many of the hydrogen-ion determinations considered in this paper, and to Irvin C. Feustel and M. S. Anderson, of the Division of Soil Chemistry and Physics, for furnishing certain materials used in the experiments.

³ Italic numbers in parentheses refer to Literature Cited, p. 633.

Hellriegel (5), the outstanding worker with sand cultures, evidently was not fully satisfied with the yields obtained in cultures of Hohenbockaer glass sand, for at some time in his 30 years' work he commenced adding 5 to 6 percent of washed and acid-treated peat to the sand. This addition is described as being made for the purpose of improving the water-holding capacity of the sand. The peat undoubtedly increased the yields but probably in some way other than through improving the water supply.

In 1915, Koch (6) obtained enormous increases, 300 percent and more, in the yields of wheat, rye, oats, and buckwheat, by mixing Zettlitzer kaolin with glass sand in the proportions of 1 to 6. He assumed that the sand-clay mixture could be more readily penetrated by roots than pure sand and attributed the increased growth to this. Ehrenberg et al. (1) in the same year reported that clay, peat, diatomaceous earth, and barytes all improved the yield when mixed with quartz sand. Like Hellriegel, they attributed the increased yields to improved water-holding capacity of the mixtures.

A year later Lemmermann et al. (7) investigated the beneficial action of the Zettlitzer kaolin in sand cultures. They disposed of the idea that it was concerned with the water supply and showed that it varied with the composition of the nutrient salts. They concluded that the kaolin, which was slightly alkaline, increased the growth of oats and buckwheat by neutralizing the acid reaction of the nutrient salts.

When studying the availability of phosphates, Lemmermann and Wiessmann (8) obtained a 50-percent increase in the yield of oats in glass sand by the addition of colloidal silica or calcium permutite, under conditions where the phosphate and other nutrients were supposedly present in excess. The beneficial action of the materials was not explained.

In the related field of water cultures there have been investigations dealing with the effects of adding such adsorptive materials as colloidal silica, humic acid, charcoal, peat, diatomaceous earth, and kaolin. The increased yields that frequently attended the use of these materials have been ascribed to the adsorption of plant toxins and heavy metals or to the maintenance of a more favorable hydrogen-ion concentration.

COMPARATIVE YIELDS IN SAND AND IN SAND-SOIL MIXTURES

Seventy-seven comparisons have been obtained of the yields of millet in quartz sand and in sand-soil mixtures, the fertilizer salts being presumably present in excess. Each sand-soil mixture contained sufficient soil to supply 1 percent of colloid. In 11 cases, involving 7 soils, yields were depressed by the soil additions; but the reasons for the depressed yields are known: Either the soils contained sufficient carbonate of lime to render iron unavailable and bring about a marked chlorosis, or they were markedly acid and fixed so much of the added potassium that yields were reduced by a potassium deficiency. In the remaining 66 cases, involving 46 different samples of soil, yields were increased by the soil applications. The increases ranged from 5 to 162 percent of the quartz-sand yields, the average increase being 56 percent.

A better yield in sand-soil mixtures than in pure sand is evidently not peculiar to millet. Both white mustard (*Brassica alba* (L.) Boiss.) and rice (*Oryza sativa* L.) in single experiments grew considerably better when soil was added to the quartz sand. On the other hand, soil additions that markedly improved the growth of millet did not appreciably affect the growth of Marquis wheat (*Triticum aestivum* L.) as shown in table 1. The values given in this table were taken from two experiments with wheat and two experiments with millet, the data of which have been published previously (4).

TABLE 1.—Wheat and millet compared as to the degree their yields are affected by addition of soil to quartz sand

Kind of soil mixed with quartz sand	Wheat, yield in soil+sand ¹	Millet, yield in soil+sand ¹
	Percent	Percent
Cecil (No. 6977).....	107	135
Muskingum (No. B407).....	102	137
Colby (No. 6842).....	103	² 52
Nacogdoches (No. 9475).....	108	171

¹ Expressed as percentage of yield in quartz sand.

² Millet developed a chlorosis induced by the calcium carbonate present in the Colby soil.

Millet plants grown in pure sand had a slightly different appearance from those grown in sand-soil mixtures and, as will be seen later, it is significant that a difference in the sizes of plants in the two mediums appeared 3 to 4 days after the plants were up. As compared with plants grown in sand-soil mixtures, the plants grown in pure sand seemed less stocky, the leaves were somewhat narrower and shorter but dark green in color, and the whole plant looked more upright and thinner. The most pronounced difference was in the roots. In the pure sand, only a root or two reached the bottom of the 1-gallon pot and the more recently formed roots were thick and crinkled. In the sand-soil mixture, the roots were thinner and much longer, forming a mat at the bottom of the pot. It seems that Koch (6) made similar observations on the root growths of plants that he grew in sand and in sand plus Zettlitzer kaolin. While there was a marked difference in the appearance of the roots, weight of roots relative to weight of tops was about the same for the two mediums in a dozen or more cases where the roots were recovered and carefully freed of sand.

In the 66 cases mentioned, where soil additions increased growth by 56 percent on the average, the quantities of soil added to the sand were sufficient to provide 50 gm. of colloid per pot, or about 1 percent of the sand-soil mixture. The effects of larger and smaller proportions of soil in the sand-soil mixture may be seen in tables 2 and 3. The values in table 2 were calculated from previously published data of experiments dealing with other subjects (3, 4). Table 3 gives the results of two experiments with the Wabash subsoil conducted at different seasons of the year.

It is apparent from table 2 that a soil application supplying 50 gm. of colloid per pot was as effective in increasing the yield as applications two to three times as large and that reducing the application to 20 or 25 gm. of colloid diminished the yield appreciably only in the case of the Kirvin soil. In the case of the Wabash subsoil (table 3), a

soil application supplying only 2 gm. of colloid per pot (0.04 percent of the mixture) was plainly beneficial, while an application supplying about 10 to 20 gm. of colloid was about the optimum. Evidently the quantity of soil required to give the best results varies with the kind of soil, just as different soils vary in the increases they produce. Also, the increase produced by one and the same soil often varies considerably in experiments run at different times. The two experiments with the Wabash subsoil reported in table 3 are merely one instance of many that have been observed. Possibly the variations were produced by differences in sunlight at different times of the year.

TABLE 2.—Yields of millet in sand-soil mixtures containing different amounts of soil relative to yield in pure sand

Kind of soil mixed with quartz sand	Yield ¹ in soil-sand mixture containing indicated amount of soil colloid per pot					
	20 gm.	25 gm.	50 gm.	60 gm.	100 gm.	150 gm.
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Chester (No. 300).....	176		170			
Kirvin A horizon (No. 6679).....	111			144		
Nacogdoches (No. 9475).....		159	185		199	
Marshall (No. 8736).....		205	202			192
Vernon (No. 6718-19).....		144	156		158	

¹ Expressed as percentage of yield in pure sand.

TABLE 3.—Comparative yields of millet in quartz sand and in different mixtures of sand and Wabash subsoil (No. 190)

Experiment and medium in which plants were grown	Air-dry yield of individual pots			Average air-dry yield per pot	
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Percent¹</i>
Experiment 25:					
Quartz sand only.....	3.54	3.50	4.42	3.82	100
Quartz sand and soil supplying 40 gm. of colloid.....	6.61	6.30	6.23	6.38	167
Quartz sand and soil supplying 20 gm. of colloid.....	6.23	6.16	6.48	6.29	165
Quartz sand and soil supplying 10 gm. of colloid.....	6.20	5.75	6.53	6.16	161
Experiment 31:					
Quartz sand only.....	2.90	2.72	2.28	2.63	100
Quartz sand and soil supplying 50 gm. of colloid.....	6.72	6.50	7.44	6.89	262
Quartz sand and soil supplying 10 gm. of colloid.....	4.98	4.98	4.84	4.93	187
Quartz sand and soil supplying 5 gm. of colloid.....	3.82	4.14	3.43	3.80	144
Quartz sand and soil supplying 2 gm. of colloid.....	4.04	2.78	2.64	3.15	120

¹ Expressed as percentage of yield in quartz sand.

THE QUANTITY AND CONCENTRATION OF SALTS AND THE WATER SUPPLY AS FACTORS IN THE INCREASED YIELD PRODUCED BY SOIL ADMIXTURES

The marked differences in yield in sand and sand plus soil could not have been due to differences in the supply of the principal nutrients. The pure sand and the sand-soil mixtures were given the same fertilization except for the phosphoric acid and the attendant calcium. Sand-soil mixtures made up with soils that render soluble phosphates unavailable were given 1½ to 2½ times as much phosphate as the pure sand cultures to insure an adequate supply. Other sand-soil mixtures containing soils found not to fix phosphates received the same amount of phosphoric acid as the pure sand. Experiments showed that the 0.20 gm. of P₂O₅ applied to the pure sand was more than was needed for maximum growth in that medium.

The quantities of fertilizer salts applied to both cultures (see table 8, fertilizer No. 2) were so large as compared with the available nutrients present in the soil additions that it seemed impossible that the sand-soil mixtures could be at any appreciable advantage over the sand cultures so far as the quantities of available nitrogen, phosphorus, potassium, calcium, magnesium, and sulfate were concerned. Also, the fact that plants in the soil-sand mixtures were larger than those in pure sand a few days after the plants were up showed that small differences in the quantities of these major nutrients could not be responsible for the different growths in the two mediums.

Early investigators attributed the increased yields they obtained with admixtures of 5 to 6 percent of peat or 16 percent of clay to an improved water-holding capacity of the medium. In view of the large quantities of absorptive material added, this does not seem an impossible explanation of their results. But it seems an absurd explanation of the results produced by the much smaller quantities of adsorptive material applied in these experiments. Most of the 46 soils that increased the yield did not sensibly alter the water-holding capacity of the sand when added at a rate to supply 1 percent of colloidal material, and even more certainly the smaller quantities of Wabash subsoil had no such effect.

The pure sand cultures and the soil-sand mixtures were made up to the same water content, about 17 percent, so that if there were a slight difference in available water it was in favor of the colloid-free sand cultures. Moreover, growth in quartz sand was not appreciably affected by marked changes in the water supply. In one experiment, water was added until it ran through a hole provided in the bottom of the pot and the leachings were subsequently used for watering. Under these conditions the sand held temporarily about 28 percent of water instead of the 17 percent normally provided, and the yield of millet was 1.81 ± 0.08 gm. per pot as compared with 1.57 ± 0.13 gm. for the normal conditions.

Related to the water supply is the concentration of nutrient salts applied. This was found not to be unfavorable in the sand cultures. At least, the early growth of millet was the same with one-half the application of salts as with the normal application. The plants, cut before the total quantity of nutrients could affect growth, weighed 0.52 ± 0.02 gm. with the normal concentration and 0.48 ± 0.04 gm. with the half-normal concentration.

TRACE ELEMENTS AS A FACTOR IN THE INCREASED YIELD PRODUCED BY SOIL ADMIXTURES

The possibility that trace elements might be responsible for the beneficial effect of soil additions was tested in a number of experiments. In an experiment previously reported (2, p. 41), the yield of millet in quartz sand was 2.10 ± 0.04 gm. per pot with the standard fertilizer and 2.18 ± 0.06 gm. with the standard fertilizer plus copper and boron. Other experiments are reported in table 4. In these experiments, conducted in quartz sand, the standard fertilizer applied was No. 2 (see table 8). The normal applications of trace elements, expressed as grams per pot, were as follows: Copper, 0.00036 gm., as cuprous chloride (Cu_2Cl_2); zinc, 0.0015 gm., as zinc sulfate (ZnSO_4); boron, 0.0015 gm., as boric acid (H_3BO_3); and manganese, 0.003 gm., as manganese sulfate ($\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$).

TABLE 4.—Yield of millet in pure sand as affected by addition of trace elements

Experiments and additions to the standard fertilizer	Air-dry yield of individual pots			Average air-dry yield per pot
	Grams	Grams	Grams	Grams
Experiment 27:				
None (check).....	2.72	2.33	2.82	2.62
Cu, Zn, Mn, B, normal rate.....	3.07	2.79	2.95	2.94
Cu, Zn, Mn, B, twice normal rate.....	2.74	3.10	2.85	2.90
Cu, Zn, Mn, B, four times normal rate.....	2.72	2.33	2.21	2.42
Cu, Zn, Mn, twice normal rate.....	2.87	2.43	2.39	2.56
Experiment 29:				
None (check).....	2.10	2.54	2.19	2.28
Tap water instead of distilled water.....	2.40	2.59	2.23	2.41
Tap water+Cu, Zn, Mn, B, normal rate.....	2.06	2.12	2.20	2.13
Experiment 58:				
None (check).....	3.52	2.80	3.68	3.33
0.0008 gm. Fl per pot from NaFl.....	3.15	3.30	2.70	3.05
0.0040 gm. Fl per pot from NaFl.....	3.00	3.28	2.78	3.02

It will be seen that trace elements, including fluorine, did not significantly affect the yields obtained in quartz sand. Evidently contamination with containers and impurities in the sand and nutrient salts provided the minute quantities needed.

AN INJURIOUS IMPURITY AS A CAUSE OF THE REDUCED YIELD IN PURE QUARTZ SAND

No evidence having been obtained that the sand cultures were deficient in a mineral nutrient, it seemed possible that an impurity in the sand that would be rendered unavailable by the soil colloids might account for growth being poorer in sand than in sand-soil mixtures. A comparison was therefore made between the ordinary quartz sand and sand purified by acid. About 60 pounds of the sand was digested for 48 hours with 10-percent nitric acid, then washed repeatedly with tap water until neutral in reaction. It was finally washed six times with 5-liter portions of distilled water. The acid evidently dissolved some iron, and the repeated washing with water floated away some very fine quartz powder. Owing to the limited quantity of purified sand available, a preliminary experiment with single pots was conducted before the rest of the sand was used in a second experiment. The results of the two experiments are given in table 5. The soils used in these experiments were applied at a rate to furnish about 1 percent of colloid to the mixture, and the standard fertilizer No. 2 (see table 8) was used. Both the sand and sand-soil mixtures received the same quantity of monocalcium phosphate in these experiments (0.27 gm. of $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ in experiment 37 and 0.36 gm. in experiment 44), since the Wabash and Marshall soils do not fix phosphates appreciably.

Plants in the purified sand became chlorotic when about half grown, but regained their green color after the addition of another dose of iron, 0.0185 gm. of ferric tartrate per pot. In the second experiment, No. 44, the chlorosis was not allowed to develop so far as in the first experiment. Evidently the poorer growth in purified sand, as compared with that in ordinary sand, was due to a temporary shortage of iron; some iron was present as an impurity in the ordinary sand. The comparative yields in the two kinds of sand, with and without the admixture of soil, gave no evidence of an injurious impurity in the ordinary quartz sand.

TABLE 5.—*Growth of millet in purified quartz sand compared with growth in ordinary quartz sand*

Experiment and medium in which plants were grown	Air-dry yield of individual pots			Average air-dry yield per pot
	Grams	Grams	Grams	
Experiment 37:				
Ordinary quartz sand.....	1.84	2.27	2.06
Ordinary quartz sand+Wabash subsoil (No. 190).....	3.64	3.58	3.61
Purified quartz sand.....	1.04	1.04
Purified quartz sand+Wabash subsoil (No. 190).....	3.67	3.67
Experiment 44:				
Ordinary quartz sand.....	2.04	1.85	1.85	1.91
Ordinary quartz sand+Marshall soil (No. 8736).....	3.53	3.31	3.52	3.45
Purified quartz sand.....	1.62	1.78	1.70
Purified quartz sand+Marshall soil (No. 8736).....	3.44	3.62	3.53

INCREASED YIELDS IN QUARTZ SAND PRODUCED BY MATERIALS OTHER THAN SOIL

Experiments were conducted from time to time to see whether the yield in pure sand could be increased by the admixture of colloidal materials other than soil. The results of these experiments, conducted in 1-gallon pots with fertilizer No. 2 (see table 8), are brought together in table 6.

The material designated as "silicic acid gel electrolyzed"⁴ had been prepared about 1 year before it was used in this study and had a pH value of 3.40 when fresh. The "silicic acid gel commercial" was a dried product of a commercial firm. The "sodium silicate neutralized" was a freshly prepared, unpurified silicic acid gel, made by dissolving sodium silicate in water and adding acid until neutral to litmus. The activated charcoal, as purchased from a commercial firm, was strongly alkaline. It was partly purified by washing repeatedly with distilled water, treating with normal sodium chloride, and then washing until the wash water had a pH value of 7.5 and gave no test with silver nitrate.

The reed peat⁵ had a pH value of 4.85. "Reed peat+CaCO₃" was the same peat to which sufficient precipitated calcium carbonate was added to give a pH value of 6.81.⁶ The iron gel was prepared by approximately neutralizing a ferric chloride solution with sodium hydroxide, washing by decantation 14 times until the gel formed a stable suspension, and then drying at 104° C. The dried material had a pH value of 8.3. All the pots in a given experiment received exactly the same fertilization except the Cecil soil-sand mixture in experiment 29, which received 0.40 gm. of P₂O₅ instead of 0.15 gm., and the iron gel in experiment 58, which received 0.50 gm. of P₂O₅ instead of 0.20 gm.

The dried silica gels, which were quite acid, did not affect the yield significantly, but the freshly precipitated gel (3½ gm. of sodium silicate neutralized) increased the yield about half as much as soil. Ten grams of peat and 10 gm. of activated charcoal gave about equal increases, which were almost as great as that produced by soil. The 10 gm. of ferric oxide gel gave an increase fully equal to that of the soil. All the materials, including the dried silica gels, induced a root growth comparable to that in the soil-sand mixtures. As previously

⁴ Prepared by M. S. Anderson.

⁵ Furnished by Irvin C. Feustel.

⁶ As determined by Irvin C. Feustel.

mentioned, in the soil-sand mixtures there was always a mat of roots at the bottom of the pot, whereas in the pure quartz sand only an occasional root reached the bottom of the pot.

TABLE 6.—*Effect of various colloidal materials on the yield of millet in quartz sand*

Experiment and special materials added to quartz sand	Air-dry yield of individual pots			Average air-dry yield per pot	
	Grams	Grams	Grams	Grams	Percent ¹
Experiment 25:					
No addition (check).....	3.54	3.50	4.42	3.82	100
2 gm. silicic acid gel electrodialyzed.....	4.06	4.29	4.79	4.38	115
4 gm. silicic acid gel electrodialyzed.....	4.02	4.14	4.36	4.17	109
10 gm. silicic acid gel commercial.....	4.40	3.97	3.64	4.00	105
2 gm. sodium silicate neutralized.....	3.47	4.23	4.02	3.91	102
Wabash subsoil (No. 190) rate 10 gm. colloid.....	6.20	5.75	6.53	6.16	161
Experiment 27:					
No addition (check).....	2.72	2.33	2.82	2.62	100
3½ gm. sodium silicate neutralized.....	2.87	3.47	3.64	3.33	127
10 gm. reed peat, pH 4.85.....	4.12	3.89	3.94	3.98	152
Marshall subsoil (No. 8737) rate 20 gm. colloid.....	4.05	4.34	3.92	4.10	156
Experiment 29:					
No addition (check).....	2.40	2.59	2.23	2.41	100
3½ gm. sodium silicate neutralized.....	3.42	3.55	3.65	3.54	147
Marshall soil (No. 8736) rate 50 gm. colloid.....	4.98	4.87	5.03	4.96	206
Cecil soil (No. 6977) rate 50 gm. colloid.....	3.57	3.80	3.49	3.62	150
Experiment 44:					
No addition (check).....	2.04	1.85	1.85	1.91	100
10 gm. activated charcoal pH 7.5.....	3.06	3.44	3.13	3.21	168
30 gm. activated charcoal pH 7.5.....	2.72	2.75	2.97	2.81	147
Marshall soil (No. 8736) rate 50 gm. colloid.....	3.53	3.30	3.52	3.45	181
Experiment 54:					
No addition (check).....	1.98	1.93	2.00	1.97	100
10 gm. reed peat.....	2.30	2.48	-----	2.39	121
10 gm. reed peat + CaCO ₃	2.43	3.13	-----	2.78	141
10 gm. activated charcoal.....	2.37	2.55	-----	2.46	125
Experiment 58:					
No addition (check).....	3.52	2.80	3.68	3.33	100
9.69 gm. Fe ₂ O ₃ gel dried.....	6.22	-----	-----	6.22	187

¹ Percentage of yield in quartz sand.

It seems reasonable to assume that these colloidal materials act in the same way as soil in increasing growth. If they do, it is plain that the increased growth produced by soil is due to no ordinary physical change in the medium. Ten grams of activated charcoal or reed peat do not appreciably affect the penetrability, coherence, pore space, or water-holding capacity of 4,800 gm. of quartz sand. Also, if the artificial additions act in the same way as soil in promoting growth, the beneficial effect of soil cannot be due to a growth-stimulating organic compound.

The materials are all adsorptive, the silica and iron gels slightly so, and the activated charcoal and peat to an equal or greater degree than soil, but it is improbable that they change the supply of nutrient ions appreciably in a favorable way. The activated charcoal, after washing, treatment with sodium chloride, and washing, would be expected to exchange only sodium and chloride with the nutrient salts applied to the sand cultures. It is possible, however, that these colloid materials do have a favorable effect on the hydrogen-ion concentration of the medium.

HYDROGEN-ION CONCENTRATION AS A FACTOR IN THE INCREASED YIELD PRODUCED BY SOIL ADMIXTURE

Many of the soils and artificial materials tested did keep the soil-sand mixtures from becoming as acid as the pure sand. Only a part of the increases the materials produced, however, could be attributed to this influence, since some soils that markedly increased growth

gave soil-sand mixtures of the same pH value as the quartz sand cultures. That the hydrogen-ion concentration may be a factor, but not the chief one, in the beneficial effect of soil in sand cultures, is indicated by comparing the pH values of different soils with the increases they produced. Ten soils that had pH values ranging from 7.87 to 6.12 increased growth by an average of 66 percent; seven soils with pH values from 6.05 to 5.52 increased growth by an average of 48 percent; and five soils with pH values of 5.25 to 4.32 increased growth by an average of 49 percent.

The quartz sand used in these experiments had a hydrogen-ion concentration of pH 6.6. The nutrient salts added to the sand and to the sand-soil mixtures are shown as fertilizer No. 2 in table 8. When these salts were dissolved in 800 cc. of water (the quantity added per pot) they gave a solution of pH 3.6. But when the salts were added to the sand the medium had a hydrogen-ion concentration of pH 6.2. After millet had been grown the sand cultures ranged from pH 3.9 to 5.7 in nine different experiments. In earlier work with a similar mixture of salts (magnesium sulfate instead of magnesium chloride and 0.535 gm. of calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) and 0.338 gm. of potassium chloride (KCl) in place of potassium nitrate) the hydrogen-ion concentration of the sand after growth of millet ranged from pH 3.9 to 5.4 in 10 different experiments. The sand cultures, therefore, became more acid with growth of millet, the hydrogen-ion concentration increasing usually from pH 6.2 to pH 5.0 to 4.5.

The effect of soil additions in modifying the acidity developed by millet in pure sand cultures is shown by the data in table 7. Column 3 shows the hydrogen-ion concentration of pure sand cultures at the end of an experiment; column 4 shows the pH values of sand-soil mixtures in the same experiment; and column 5 shows the pH values of the soils used in making up the sand-soil mixtures.

TABLE 7.—Hydrogen-ion concentrations developed by millet in sand and in sand-soil cultures, pH values of soils used in sand-soil mixtures, and yield in sand-soil mixtures relative to yield in pure sand

Experi- ment No.	Kind of soil mixed with the sand	Hydrogen-ion concentration of—			Yield in sand-soil mixture ¹
		Pure sand culture	Sand-soil culture	Soil used in sand- soil cul- ture	
		pH	pH	pH	Percent
5	Barnes, No. 10305.....	3.9	6.4	7.2	190
5	Miami, No. 10341.....	3.9	6.7	6.9	177
5	Davidson, No. 4429.....	3.9	6.2	6.4	189
6	Carrington soil, No. 10082.....	5.0	4.8	5.5	179
6	Clarksville soil, No. 195.....	5.0	5.0	5.4	121
6	Clarksville subsoil, No. 196.....	5.0	4.8	4.9	167
7	Nacogdoches, No. 5028.....	4.8	6.3	6.3	164
9	Kirvin soil, No. B299.....	5.0	5.7	6.1	148
10	Norfolk soil, No. 183.....	4.5	4.7	4.8	161
10	Norfolk subsoil, No. 184.....	4.5	4.3	4.3	125
10	Vernon 0-3, No. 6718.....	4.5	6.1	7.6	153
10	Vernon 3-10, No. 6719.....	4.5	6.1	7.0	209
10	Vernon 10-27, No. 6720.....	4.5	6.1	6.4	201
37	Wabash subsoil, No. 190.....	5.0	5.9	5.8	175
44	Marshall soil, No. 8736.....	5.8	6.2	6.4	181
60	do.....	5.5	6.0	6.2	143
61	Marshall soil, No. 8736, heated.....	5.5	5.1	5.6	198

¹ Expressed as percentage of yield in pure sand.

It will be seen that in sand-soil mixtures the hydrogen-ion concentration is stabilized around that of the soil used in making the mixtures. In most cases the sand-soil mixtures are less acid than the sand culture in the same experiment. But in seven cases the two mediums have nearly the same pH value, and in five of these cases the increased yield produced by the soil admixture is almost as great as when the soil-sand mixture is less acid than the pure sand culture. It seems, then, that regulation of the hydrogen-ion concentration is not the chief factor in the increase produced by mixing soil with quartz sand.

YIELDS IN SAND AND IN SAND-SOIL MIXTURES WITH FERTILIZERS GIVING DIFFERENT HYDROGEN-ION CONCENTRATIONS

Further evidence as to whether the changes in hydrogen-ion concentration could account for the increased growth in soil-sand mixtures was obtained in two experiments. In these experiments comparisons were made of six different fertilizer mixtures that would induce different hydrogen-ion concentrations in the sand cultures. The important variable in the fertilizer mixtures was the ammonium ion, this being chiefly responsible for the acidity developed.

The compositions of the fertilizer mixtures applied are shown in table 8. All mixtures were the same with respect to the quantities of nitrogen, phosphorus, potassium, magnesium, sodium, iron, and manganese present. The quantities of sulfate, chlorine, and calcium varied in some mixtures. The first four mixtures differed from one another chiefly in the relative amounts of the NH_4 and NO_3 radicles. Fertilizers 5 and 6, containing all the nitrogen as NO_3 , were nearly alike except that No. 5 contained a neutral mixture of phosphates, while No. 6 contained the alkaline dipotassium phosphate only. No. 2 was the standard mixture used in the experiments reported previously.

Detailed results of the two experiments in which the fertilizer mixtures were used are given in table 9. The same Marshall soil, No. 8736, was used in the two experiments, but in experiment 61 it was heated for 24 hours at 105°C . to kill any root rot organisms that might be present. The unheated soil of experiment 60 had a pH value of 6.15; the heated soil of experiment 61 had a pH value of 5.62. In the treatment "Marshall soil in layers," experiment 61, the soil was mixed with only 10 percent of the sand and this mixture was placed in the pot in four thin layers that were separated by pure sand. The total volume of the layers was about 13 percent of the whole volume of sand in the pot.

In experiment 60 the plants in pure sand receiving the all-nitrate fertilizer No. 6 became strongly chlorotic. In experiment 61, therefore, an extra series labeled "extra Fe" was installed with all-nitrate fertilizer No. 5 to receive extra iron if chlorosis developed. The plants in pure sand receiving this fertilizer did become chlorotic 12 days after planting, and these plants, as well as those in the sand-soil mixture, which were not chlorotic, were then given a dose of 0.0185 gm. of ferric tartrate and 2 days later another dose of 0.0185 gm.

In experiment 60, both the pure sand with fertilizer No. 2 and the sand-soil mixture with fertilizer No. 1 had a pH value of 5.5 after growth and the sand-soil mixture gave a much larger yield (49 percent more). Likewise the sand-soil mixture with fertilizer No. 2 and the pure sand with fertilizer No. 3 had practically the same pH value,

but the sand-soil mixture yielded 37 percent more. In experiment 61, the sand-soil mixture with fertilizer No. 2 had a pH value of 5.1, as compared with pH 5.5 for the pure sand but gave a yield 98 percent greater than the pure sand. These results substantiate the conclusion that change in the hydrogen-ion concentration is only a minor factor in the increases in yield produced by adding soil to sand cultures.

TABLE 8.—Composition of fertilizer mixtures used in experiments

Salt used in fertilizer mixtures	Amount of salt applied per pot in fertilizer—					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>	<i>Gram</i>
Potassium nitrate, KNO_3		0.93	0.93	0.93	0.499	0.414
Ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$33				
Ammonium nitrate, NH_4NO_3	0.57		.20	.10		
Calcium nitrate, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$304	1.103	1.16
Potassium sulfate, K_2SO_482					
Magnesium sulfate, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$45	.45	.45	.45
Magnesium chloride, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$42	.42				
Monocalcium phosphate, $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$36	.36	.36	.36		
Dipotassium phosphate, K_2HPO_425	.49
Monopotassium phosphate, KH_2PO_419	
Sodium chloride, NaCl05	.05	.05	.05	.05	.05
Ferric tartrate, $\text{Fe}_2(\text{C}_4\text{H}_4\text{O}_6)_3 \cdot \text{H}_2\text{O}$0185	.0185	.0185	.0185	.0185	.0185
Manganese sulfate, $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$0015	.0015	.0015	.0015	.0015	.0015

TABLE 9.—Yields of millet in sand and in a Marshall soil-sand mixture¹ with different fertilizers²

Experiment and medium in which plants were grown	Air-dry yield of individual pots					Reaction of medium after growth
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	
Experiment 60:						
Fertilizer No. 1+pure sand.....	2.39	2.18	1.75	2.25	2.14	4.3
Fertilizer No. 1+sand+Marshall soil.....	3.45	3.60	3.82	4.48	3.84	5.5
Fertilizer No. 2+pure sand.....	2.69	2.65	2.31	2.66	2.58	5.5
Fertilizer No. 2+sand+Marshall soil.....	3.35	4.18	2.88	4.29	3.68	6.0
Fertilizer No. 3+pure sand.....	2.58	2.56	2.80	2.78	2.68	5.8
Fertilizer No. 3+sand+Marshall soil.....	4.95	4.50	3.35	4.30	4.28	6.8
Fertilizer No. 6+pure sand.....	2.15	1.98	1.62	1.97	1.53	8.0
Fertilizer No. 6+sand+Marshall soil.....	4.25	4.05	3.67	4.42	4.10	6.8
Experiment 61: ¹						
Fertilizer No. 2+pure sand.....	3.27	3.92	3.07	3.51	3.44	5.5
Fertilizer No. 2+sand+Marshall soil.....	6.69	6.81	6.53	6.95	6.75	5.1
Fertilizer No. 2+sand+Marshall soil in layers.....	7.35	6.34	7.08	6.95	6.93	5.1
Fertilizer No. 4+pure sand.....	5.40	4.40	5.10	4.30	4.80	6.8
Fertilizer No. 4+sand+Marshall soil.....	7.53	6.86	6.35	6.95	6.92	5.9
Fertilizer No. 5+pure sand.....	2.51	2.62	1.08	2.17	2.10	7.1
Fertilizer No. 5+pure sand+extra Fe.....	3.07	3.21	2.90	4.65	3.46	7.1
Fertilizer No. 5+sand+Marshall soil.....	7.55	7.25	7.33	7.68	7.45	6.4
Fertilizer No. 5+sand+Marshall soil+extra Fe.....	7.17	6.98	6.25	6.18	6.65	6.6

¹ Marshall soil was used at the rate of 50 gm. of colloid per pot. In experiment 61 it was heated for 24 hours at 105° C.

² For composition of each fertilizer, see table 8.

The influence of hydrogen-ion concentration on yields in pure sand and in the Marshall sand-soil mixture is shown in table 10, the values for which were calculated from the results of experiments 60 and 61, reported in table 9. Since data obtained in the two experiments may not be exactly comparable, especially in the case of the sand-soil mixture, the Marshall soil having been heated in experiment 61, the values based on experiment 61 are given in parentheses to distinguish them from values based on experiment 60.

TABLE 10.—*Influence of hydrogen-ion concentration on yields of millet in pure sand and in a sand-soil mixture*

Experiment from which data were obtained (No.)	Fertilizer applied	Ammonia in fertilizer ¹	Pure sand		Sand+Marshall soil	
			Yield ²	Hydrogen-ion concentration after growth	Yield ²	Hydrogen-ion concentration after growth
	No.	Mol.	Percent	pH	Percent	pH
60.....	1	0.00713	83	4.3	104	5.5
60.....	2	.00500	100	5.5	100	6.0
61.....	2	.00500	(100)	(5.5)	(100)	(5.1)
60.....	3	.00250	104	5.8	116	6.8
61.....	4	.00118	(137)	(6.8)	(100)	(5.9)
61.....	5	None	(60)	(7.1)	(107)	(6.4)
60.....	6	None	75	8.0	111	6.8

¹ Expressed as mols NH₄ per pot.² Expressed as percentage of yield obtained with fertilizer No. 2.

Columns 3 and 5 show that the acidity developed in quartz sand is dependent on the quantity of ammonia in the fertilizer. This is also apparent in the sand-soil mixture if the data of experiments 60 and 61 are considered separately.

In the pure sand culture series there is a measurable reduction in yield at pH 4.3 from that obtained at pH 5.5 to 5.8 and a marked increase at pH 6.8. The reduced yields at pH 7.1 and 8.0 are due largely to an iron deficiency produced by the low availability of iron at these hydrogen-ion concentrations. Had iron and phosphate been kept available, yields might have increased at hydrogen-ion concentrations less than pH 6.8. This is shown by the yields of fertilizer No. 5 with "extra Fe" (table 9).

The spread in yields between pH 4.3 and pH 6.8 in the sand cultures is equal to only about one-half the increases produced by soil additions. It therefore seems that regulation of the hydrogen-ion concentration is not the chief factor in the increases produced by mixing soil with quartz sand.

In the sand-soil mixture there is less spread in pH values owing to the buffering effect of the soil colloids. But it also appears as though a given difference in hydrogen-ion concentration is attended by a smaller difference in yields in the sand-soil mixture than in the pure sand. In the pure sand cultures of experiment 61, a 37-percent difference in yield accompanied a change in hydrogen-ion concentration from pH 5.5 to pH 6.8; whereas in the sand-soil mixture, only a 7-percent difference in yield accompanied a change in hydrogen-ion concentration from pH 5.1 to pH 6.4.

SUGGESTED EXPLANATION OF THE INCREASED YIELDS PRODUCED BY MIXING SOIL WITH QUARTZ SAND

If the yield of millet in pure sand falls off more rapidly than the yield in sand-soil mixtures as the hydrogen-ion concentration is increased from near neutrality, as table 10 indicates, the difference in yield between the two mediums should be less near the neutral point than at pH 4.5 to 5.0. Data given in table 9 indicate that this is the case. When the fertilizer applied was No. 4, which gave a pH value of 6.8 in pure sand, the sand-soil mixture yielded 44 percent more than

the pure sand; whereas when the fertilizer applied was No. 2, which gave a pH value of 5.5, the sand-soil mixture yielded 98 percent more than the pure sand.

Furthermore, near the neutral point the smaller increases produced by soil might reasonably be attributed to soil colloids providing available iron in the presence of available phosphates. Millet in pure sand cultures seems to be on the border line of an iron deficiency around pH 6.8, although there does not seem to be a deficiency of iron in plants growing in sand-soil mixtures. This was evident in experiments 60 and 61 (table 9). Here the plants in pure sand receiving fertilizer No. 4 showed a faint chlorosis at times, whereas the plants in sand-soil mixtures did not. Also, there was a marked chlorosis and a greatly reduced yield in the pure sand with fertilizer No. 5, which developed a pH value of 7.1.

Even granting that different yields in the two mediums might have been explained on the basis of iron availability had experiments been conducted with a fertilizer developing a neutral reaction, this still would not explain the 66 cases that have been mentioned where soil admixtures produced an average increase of 56 percent. In these cases, fertilizer No. 2 was used and the hydrogen-ion concentration developed in the pure sand cultures ranged from pH 3.9 to 5.7. At these hydrogen-ion concentrations there was no iron deficiency in the sand cultures, as shown by the dark green of the leaves.

It is believed that in the cases where fertilizer No. 2 was used the roots in the sand-soil mixture were in a less acid environment than in the pure sand cultures and that differences in growth in the two mediums were due to this difference in acidity. This hypothesis is in direct contradiction to conclusions previously drawn concerning the influence of the hydrogen-ion concentration. And it seems contradicted by several instances where the sand-soil mixtures had the same hydrogen-ion concentration as the pure sand cultures. It should be borne in mind, however, that the hydrogen-ion concentrations that have been considered were those of the whole volumes of sand or of sand and soil in the pot, and these data may not be directly pertinent.

Of course it is not the hydrogen-ion concentration of the whole medium that affects the root, but the hydrogen-ion concentration in the water film immediately contiguous to the root. We have no means of measuring the acidity of this film, but presumably it is much higher than that of the whole mass of sand since it is in this film that acidity is developed, following the more rapid absorption of ammonium ions than of nitrate ions. There is some evidence of a higher acidity adjacent to the roots in experiment 61 (table 9), where the soil was applied in four $\frac{1}{4}$ -inch layers separated by $1\frac{1}{2}$ inches of pure sand. The root development was largely concentrated in the thin sand-soil layers. The sand-soil layers and the pure sand layers were sampled separately in some pots, and the hydrogen-ion concentrations were as follows: Sand-soil layers, pH 4.6; sand layers, pH 5.1; and all the material in the pot, sampled as usual, pH 5.02.

Of course, in sand-soil mixtures, as well as in pure sand, the acidity in layers contiguous to the roots should be greater than that of the whole medium; but owing to the buffering effect of soil colloids, the hydrogen-ion concentration of the contiguous sand-soil layers should be less than that of the sand layers.

The hypothesis suggested—that the beneficial effect of mixing soil with sand is due to a reduced acidity in films contiguous to the roots when a fertilizer developing acid is used and is due to the iron supply being maintained when a fertilizer developing alkali is used—is in accord with the experience gained in growing plants in water cultures. A markedly acid reaction depresses growth in water cultures, and when the reaction approaches neutrality growth is likely to be depressed by a deficiency of iron or phosphates. Water cultures and sand cultures differ, however, in that in water cultures, owing to movement of the medium and free diffusion of ions, there is no opportunity for a markedly different layer to develop adjacent to the roots. In sand cultures, it is presumable that a hydrogen-ion concentration developing from an unequal absorption of ions by the roots may, near the roots, temporarily approach the extreme attainable by this process.

SUMMARY

This paper reports the results of an investigation dealing with the growth made by millet in pure quartz sand as compared with the growth made in sand-soil mixtures, the fertilizer salts presumably being in excess in both mediums.

In 66 cases, involving 46 soil samples, millet yielded 56 percent more, on an average, in sand-soil mixtures containing 1 percent of soil colloids than in pure quartz sand. Wheat, however, in 4 comparisons, gave about the same yield in both mediums.

The beneficial effect of soil admixtures on the yield of millet did not increase as the quantity of soil added was increased above that supplying 1 percent of soil colloids. A soil admixture supplying less than 0.2 percent of soil colloids markedly increased the yield.

The greater yield in sand-soil mixtures did not seem to be due to an effect of soil on the water-holding capacity of the medium nor on the concentration of nutrient salts.

The beneficial effect of soil admixtures seemed not to be due to supplying the trace elements copper, manganese, zinc, boron, or fluorine.

The comparatively low yield of pure sand cultures was not due to the presence of an impurity in the sand extractable in 10-percent nitric acid.

Small quantities of peat, activated charcoal, iron gel, and freshly precipitated silica gel, when mixed with quartz sand, produced an increase in yield and a modification in root growth similar to those produced by soil.

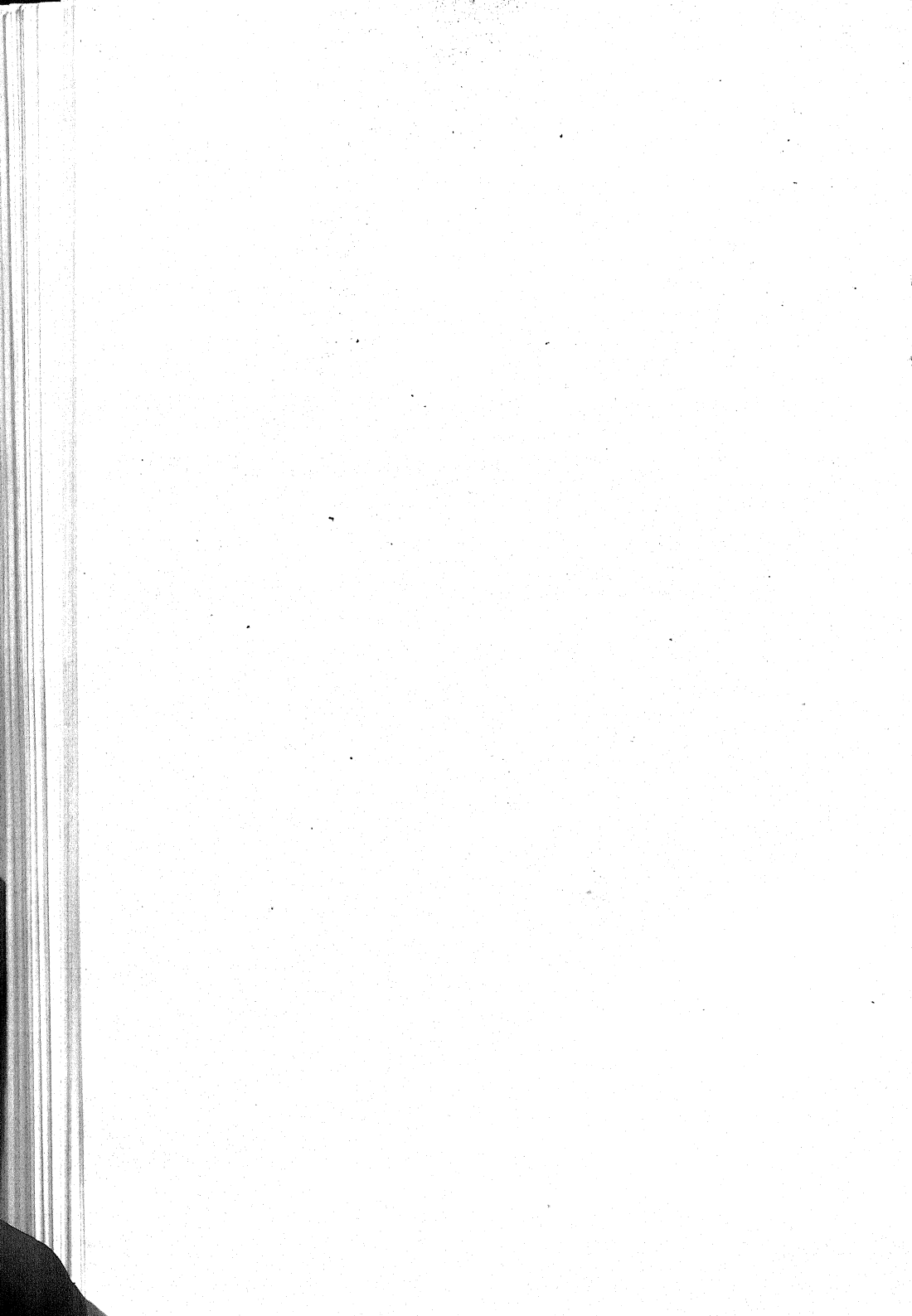
Determinations of the hydrogen-ion concentrations of the cultures, after growth and experiments with fertilizers developing different degrees of acidity owing to different proportions of ammonium and nitrate ions, indicate that the beneficial effect of soil does not lie in modifying the hydrogen-ion concentration of the whole medium.

It is suggested, however, that the hydrogen-ion concentration affecting growth is that of the water films immediately contiguous to the roots and that this is probably not shown by a sample of the whole medium. It is probable that when a physiologically acid fertilizer is used an injurious acidity is developed in sand cultures in films contiguous to the roots. In sand-soil mixtures the films are

doubtless less acid, owing to the buffering effect of soil, and the yield is consequently greater. When a physiologically neutral fertilizer is used, sand-soil mixtures should yield more than pure sand, owing to the effect of soil colloids in maintaining the availability of iron.

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COMPARATIVE STUDY OF THE APPLE ANTHRACNOSE AND PERENNIAL CANKER FUNGI¹

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INTRODUCTION

The closely related apple-tree cankers, apple anthracnose, caused by *Neofabraea malicorticis* (Cordley) Jackson, and perennial canker, caused by *Gloeosporium perennans* Zeller and Childs, are native to the Pacific Northwest. The geographic ranges of the two overlap to some extent, but generally in the districts where one is abundant the other is either absent or rare.

A study of these two diseases and their causal agents during the past 6 years has shown them to be similar in so many respects that identification has sometimes been uncertain or impossible. The fungi, as described from their typical development, are quite distinct, but in the regions where both occur the definite identification of intermediate types becomes a difficult problem.

Previous studies have been devoted almost entirely to practical methods of control, and the etiological phases have been greatly neglected. Since a preliminary study of the previously unreported ascigerous form of the perennial canker organism had shown it to be very similar to *Neofabraea malicorticis*, a comparative study of the two fungi was undertaken and the results are reported in this paper.

LITERATURE REVIEW³

The literature of apple anthracnose has been summarized to recent date in books by Heald (20, pp. 500-511)⁴ and by Owens (33, pp. 288-299). The reader is referred to these standard texts for detailed historical information.

The apple anthracnose fungus (*Neofabraea malicorticis*) first attracted attention as a serious orchard parasite about 1891. Early investigations (7, 9) proved the disease to be due to a parasitic fungus. The imperfect or acervular stage of this organism was described from Oregon by Cordley (7, 8, 9) as *Gloeosporium malicorticis*, and at about the same time by Peck (34, p. 21) as *Macrophoma curvispora* from specimens submitted to him from British Columbia. The former determination was confirmed by Lawrence (27), although in a later article (28, pp. 32-33) he used the name *Myxosporium curvisporum*.

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² Special appreciation is expressed to Dr. John W. Roberts, of this Division, and to Dr. S. M. Zeller, of Oregon State College, under whose supervision the work was carried on. Leroy Childs, superintendent of the Hood River branch station of the Oregon Agricultural Experiment Station; Dr. H. R. McLarty, of the Dominion Experimental Farms, Summerland, British Columbia; and E. L. Reeves, of this Division, gave helpful suggestions during the work. Other investigators, both local and Canadian, were helpful in various ways. The writer is grateful for the help received from these workers.

³ A complete bibliography of the literature dealing with the apple anthracnose and perennial canker diseases appears in the author's thesis. See footnote 1.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 663.

(Peck) Sacc. The ascigerous stage was discovered by Jackson (24) in 1909, for which he established the new genus *Neofabraea* and named *N. malicorticis* (Cordley) Jackson as the type species.⁵

A similar canker disease was distinguished by Zeller and Childs (41, 42) in 1925, although it was undoubtedly present in the Pacific Northwest long before this discovery (14). It was found that this "false anthracnose" was not controlled by the copper sprays effective against the common apple anthracnose. The disease was termed "perennial canker" because of the apparently perennial character of the cankers, and the causal organism was described as *Gloeosporium perennans* Zeller and Childs (42). Infections occurred through wounds of various kinds, whereas apple anthracnose resulted from infections through apparently sound bark. The perennial canker fungus produced straight to slightly curved rather than curved or hooked conidia, and showed less diastatic power on certain starchy media. McLarty (29) has reviewed the perennial canker literature and has presented the facts as they are commonly accepted at the present time.

Miller (30) gives results of a special comparative study with these fungi. He states that in his tests both organisms reacted in a similar way. Although certain differences were apparent with different cultures, more variation existed between the various strains of either species than between the species themselves. Introduction of tannic acid in the culture medium, however, appeared to inhibit the average growth rates of all strains of *Neofabraea malicorticis* to a greater degree than those of *Gloeosporium perennans*. Similar results were reported in a brief abstract by the writer (25), when malachite green was used as the inhibitory agent.

In previous work several characters have been reported by which one supposedly should be able to distinguish apple anthracnose from perennial canker (fig. 1, A and B). The most important of these are as follows:

(1) Anthracnose usually occurs west of the Cascade Range; perennial canker is most common east of this mountain range (6, 21, pp. 13-17).

(2) Copper fungicides applied before fall rains prevent anthracnose infections but have little influence upon perennial canker⁶ (5, 13, 14, 36).

(3) The apple anthracnose fungus infects apparently sound bark, often through lenticels, and outbreaks are independent of the woolly apple aphid and low temperatures (5, 29). Perennial canker infections occur only through evident injuries to the host. Galls produced on callus tissue by the woolly apple aphid (*Eriosoma lanigerum* (Hausmann)), which rupture at low temperatures, form the usual infection courts under natural conditions.

(4) Conidia from anthracnose cankers vary in shape from curved to "hooked"; those of perennial canker vary from straight to slightly curved. Lawrence (27) and Zeller and Childs (42) report that anthracnose conidia from apple fruit rots and in culture show curvature, whereas perennial canker conidia grown in the same way are relatively straight.

⁵ Nannfeldt (31) transferred the genus *Neofabraea* to *Pezizula*, based upon the European species *N. corticola* (Edg.) Jorg., a species typical of the genus *Pezizula*. Drs. J. Walton Groves and G. E. Thompson have compared the genera and found them to be distinct. A notice of the error appears in a recent paper by Thompson (40).

⁶ BARRS, H. P. NORTHWESTERN APPLE TREE ANTHRACNOSE CANKER AND FRUIT ROT (*NEOFABRAEA MALICORTICIS*). Oreg. Agr. Col. Ext. Cir. 220, 3 pp. 1925. [Mimeographed.]

(5) The anthracnose fungus shows a greater diastatic activity on certain starchy media than the perennial canker organism (42).

(6) A perfect stage has been previously reported only for the anthracnose fungus (24).

(7) Fruit rots caused by *Neofabraea malicorticis* tend to show slightly more zoning or more concentrically ringed effects than fruit rots caused by *Gloeosporium perennans*, and minor differences appear in sporulation characters (11, 12, 13, 42).

(8) Observations indicate that old anthracnose cankers tend to present a "fiddle-string" appearance, presumably because of failure of

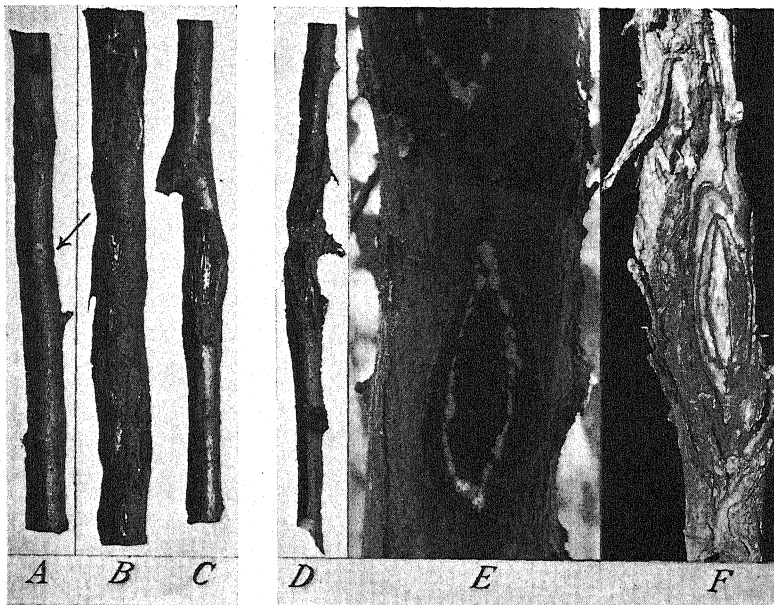


FIGURE 1.—Common sequence of the apple anthracnose and perennial canker diseases in orchards. A, B, C, Apple anthracnose: A, Primary infections through apparently sound bark, indicated by arrow; B, numerous infections developing below an old canker; C, twig showing eventual healing of a wound, which takes place unless the limb is girdled or callus tissue becomes injured and reinfected with perennial canker. D, E, F, Perennial canker: D, Primary infections through wounds; E, callus tissue injured by feeding of the woolly apple aphid, thus forming galls that rupture at low temperatures and become infected; F, perennial types of cankers resulting from annual reinfections of injured callus tissue.

the fungus to attack the bast fibers. Perennial cankers rarely show these exposed fibers.

DISTRIBUTION AND OCCURRENCE

The comparative distribution of the apple anthracnose and perennial canker fungi, based upon a survey of the literature, examinations of cankered specimens, and letters from workers in the various districts, is illustrated in figure 2.

Anthracnose seems, as a rule, to be peculiar to those humid regions of the Pacific Northwest that have a moderately high rainfall and mild winter temperatures (6). In general, this includes the humid division

of the Transition life zone (1), lying mostly west of the Cascade Range and having an average annual precipitation of approximately 35 to 80 inches. The disease is comparatively rare in northern California, common in western Oregon, western Washington, and British Columbia, and probably reaches its northern limit near Prince Rupert, British Columbia. A single report as far east as Nebraska was given by Heald (20). Hilborn⁷ very recently reported the presence

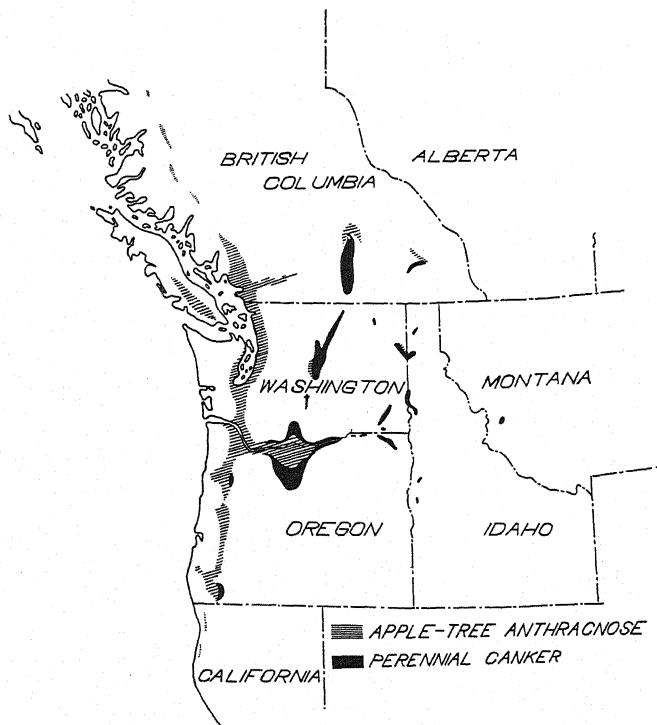


FIGURE 2.—Comparative distribution of the apple anthracnose and perennial canker diseases in the Pacific Northwest.

of anthracnose in Maine. The anthracnose organism has been reported as occurring on pear and apple trees in Denmark (16) and Netherlands (35).

Perennial canker occurs most often east of the Cascade Range in the drier Transition and Sonoran zones, characterized by lower winter temperatures and usually with less than 25 inches of annual precipitation. Its range extends from southern Oregon to the northern end of the Okanagan Valley in British Columbia and from the Cascade Range to western Montana. Brien (3, 4) reported this canker organism as causing a fruit rot in New Zealand.

In the White Salmon-Hood River Valley area of Washington and Oregon, anthracnose considerably overlaps the range of perennial canker. The Columbia River breaks through the Cascade Range at this point, and climatic conditions are intermediate between those of

⁷ HILBORN, M. T. NORTHWESTERN APPLE TREE ANTHRACNOSE FOUND IN MAINE. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22: 354. 1938. [Mimeographed.]

the humid coastal region and the semiarid interior. A similar condition occurs in the Okanagan Valley of British Columbia. There is an overlapping of the diseases near Armstrong, where the precipitation is greater than that of the lower Okanagan Valley, in which perennial canker is dominant. Occasionally there are reports of the finding of anthracnose cankers in perennial canker areas. It is to be noted that such specimens are generally found in the mountainous foothill sections where precipitation increases and other climatic factors are different from those of the bordering perennial canker area. The rare appearance of perennial canker in regions where anthracnose is of common occurrence appears to be correlated with unusually severe winter temperatures.

An examination of the conidia of *Neofabraea malicorticis* produced in cankers from the above areas impresses one with the variation in spore shape exhibited by the fungus. Conidia from the occasional anthracnose cankers found east of the Cascade Range, or those from the intermediate areas, often appeared identical with, or even less curved than, the *Gloeosporium perennans* conidia in the more humid sections. Conidia in perennial cankers from the dry sections were practically straight, whereas considerable curvature might be present in those from the overlapping range. A somewhat progressive increase in degree of curvature of the conidia appeared to occur from the eastern to the western limits, so that in the strictly coastal sections anthracnose conidia were decidedly curved or "hooked." While this general trend was apparent, exceptions were frequent. Besides this regional variation, conidial shapes of known strains varied somewhat from season to season. The difficulty often encountered in identifying the two organisms from the size or shape of the conidia is apparent in figure 3, where typical conidia are illustrated in comparison with some intermediate regional forms and with the usual types of conidia produced in cultures and on apple rot tissue under Hood River conditions. Host variety seemed to have less influence on conidial curvature than did type of tissue infected or local environmental conditions.

HOST RELATIONS

The anthracnose and perennial canker fungi attack similar host plants (19, 25, 36, 41). The former infects young trees and bark more commonly than well-matured trees, and in general, early-maturing varieties of apples are less susceptible than fall sorts. However, very few definite data are available concerning varietal resistance. Resistance to the perennial canker disease among apple varieties corresponds to their winter hardiness and freedom from woolly aphids. Individuals of a single variety may vary in these respects according to their vigor or because of other complex factors. *Gloeosporium perennans* was reported to have been isolated from cherry buds dying during the winter in Oregon (32), but neither fungus is commonly encountered on stone-fruit trees.

Shear and Cooley (38) showed by inoculations that trees have a definite period of susceptibility to the perennial canker organism. Susceptibility increased rapidly from a low point during September, reached a maximum in November and December, and subsided to infrequent infections by the middle of January. McLarty (29) proved that without freezing injury to the tissue the fungus was unable to

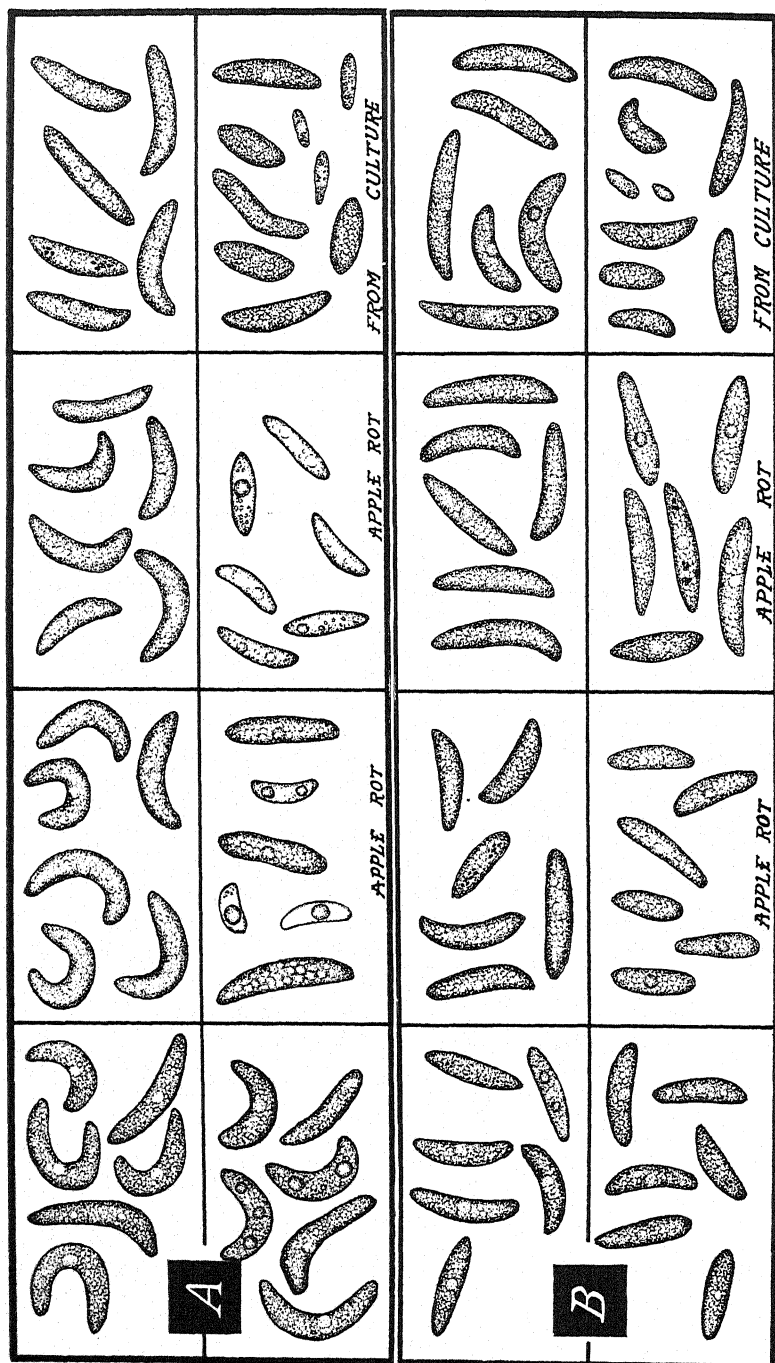


FIGURE 3.—Variation in conidial shapes of *Neofabraea malicorticis* and *Gloeosporium perennans*: A, *N. malicorticis*; B, *G. perennans*. Typical conidia are shown at the extreme left in each section, and intermediate forms from the overlapping geographical ranges are shown to the right. Types formed on apple rot tissue and in culture at Hood River are indicated. Approximately $\times 450$.

infect the host, even when it was inoculated into the tree during the susceptible period. The detailed behavior of the anthracnose fungus in relation to periods of host susceptibility has not been reported, but it appears to be similar to perennial canker.

It was thought possible that parallel inoculations with anthracnose and perennial canker fungi into various host species might yield some distinctive characters of taxonomic value. Accordingly, 1 typical strain of each fungus was inoculated into 12 genera of the Rosaceae, considered in its broadest sense, and into 30 species in 10 other families. Cankers developed from inoculations with each fungus on the following rosaceous hosts: Apple (*Malus sylvestris* Mill.), Siberian crab (*M. baccata* Borkh.), Oregon crab (*M. rivularis* Roemer), pear (*Pyrus communis* L.), quince (*Cydonia oblonga* Mill.), peach (*Amygdalus persica* L.), serviceberry (*Amelanchier pallida* Gr.), apricot (*Prunus armeniaca* L.), plum (*Prunus* sp.), cherry (*P. avium* L.), pin cherry (*P. emarginata* Walp.), flowering quince (*Chaenomeles* sp.), hawthorn (*Crataegus* sp.), and the wild and cultivated mountain-ash (*Sorbus* spp.)

None of the cankers on stone-fruit trees was typical of the diseases as they occurred on pome-fruit trees. Neither acervuli nor spores were produced and it was considered doubtful that cankers on these stone-fruit species could form naturally. The cankers found by Lawrence (27) were probably caused by different parasites, especially the bacterial gummosis organism *Phytophthora syringae* (Griffin) Bergey et al. (*P. cerasi* Griffin) in the case of cherry and prune.

Cankers similar to those occurring naturally on apple trees were produced by inoculations into the native Oregon crab, serviceberry, and mountain-ash. A statement by Pierce, reported by Jackson (24), that he had found the plant that probably is the native host of the anthracnose organism, is of interest here. The native Oregon crab might be that host plant, since it is known to range throughout the Pacific coast region in proximity to many orchards. Cankers produced on it by inoculations and those found occurring naturally are similar in all respects to those on apple trees.

Results of comparative inoculations into these three native species have been obtained. The data indicated that wild host plants have a maximum period of susceptibility similar to that in apple trees, but they appeared to exhibit little selective action toward the two fungi.

THE FUNGI IN CULTURE

EFFECT OF HYDROGEN-ION CONCENTRATION AND TEMPERATURE

Miller (30) reported that *Neofabraea malicorticis* and *Gloeosporium perennans* were very similar in their growth responses to temperature or to pH values of culture media. Both fungi exhibited increased growth with rise of temperature in the range of 0° to 20° C., whether inoculated into apple fruits or artificial media. He also reported that initial growth failed to occur in media at pH values of 2.0 and 12.0, while a definite retardation was apparent at 3.0 and 11.8. There was little difference between the growth of the two organisms at a given pH value, and greater variation often occurred between the different strains of a species than between the species themselves.

Touzeau⁸ obtained similar results in a study of the influence of these two factors upon the growth of the two canker organisms, and his results have been substantiated in a general way by the writer. The pH values and temperature ranges offer conditions under which the various strains of the fungi may show considerable variation, but where constant distinctions between the two species fail to appear.

STANDARD MEDIA

GENERAL CONSIDERATIONS

More than 40 geographic or cultural strains of these organisms have been used in various experiments. They have been cultivated on a large number of media, including the standard types and many special kinds. Striking variations occurred between cultures growing on ordinary media, but these were more often intraspecific than interspecific in character. In general, when colored mycelia formed in agar cultures there was a tendency, in the majority of cases, for *Neofabraea malicorticis* to vary from white to true pinks and reds and for *Gloeosporium perennans* to vary from white to orange pinks and browns. Either organism commonly produced olive and darker pigments or mixtures of several colors. Some of these differences can be visualized from figure 4, but only a rough idea of the color variations can be presented. Characters to separate the species by simple cultural methods were not discovered.

COLOR PRODUCTION

Cultures known to produce highly colored mycelia on certain media failed to do so when the substratum was lacking in carbohydrate, nitrogen, oxygen, or light supply. A single exposure to light for a few minutes induced the formation of colored mycelia. Compatible bacterial contaminants and some fungi usually induced formation of red pigment at the point of contact.

CONIDIAL FORMATION

Typical macroconidia, as found in natural bark cankers, rarely formed in culture. When these did occur it was usually in cultures recently isolated, those transferred to radically different media, or in old tube cultures. Conidia varying in size and shape but generally smaller than true macroconidia were frequently produced in culture and are illustrated in figure 3, A and B. These were considered to be immature macroconidia, modified by cultural conditions, since in other respects they functioned as true conidia.

Microconidia are more often formed, especially from mycelia produced by macroconidia or ascospores germinated directly in water or from mycelia growing on media deficient in carbohydrates. In some cases these appeared to be budded off from macroconidia or from ascospores directly, but usually they were produced on short sterigmata, as in figure 5, D. This condition has been illustrated by Zeller and Childs (42).

⁸ TOUZEAU, WALTER D. A CULTURE COMPARISON OF GLOEOSPORIUM PERENNANS ZELLER AND CHILDS AND NEOFABRAEA MALICORTICIS (CORDLEY) JACKSON. Unpublished thesis, Univ. of Brit. Columbia. 1934.

HYPHAL COILS AND CELL FUSIONS

Several interesting structures have been observed in cultures on plain agar or in water where it was possible to follow the development of the mycelium. The young hyphae frequently became coiled at intervals, and when this occurred microconidia usually appeared on

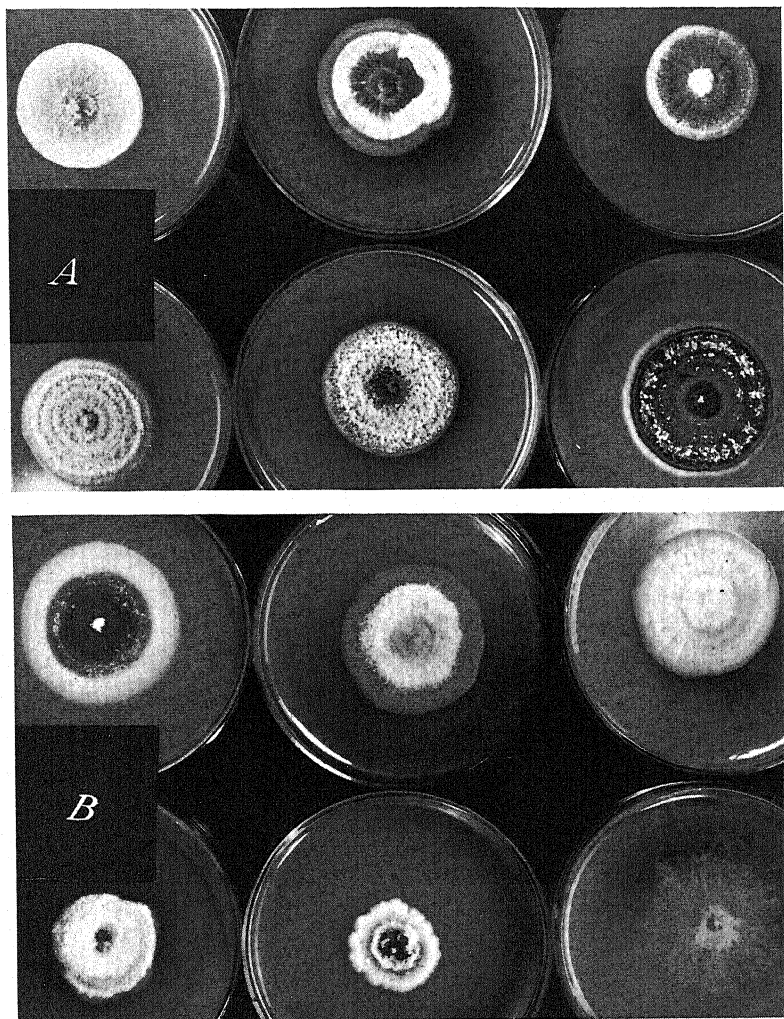


FIGURE 4.—Appearance of six strains of *Neofabraea malicorticis* (A) and six strains of *Gloeosporium perennans* (B), grown 20 days on Leonian's medium B at room temperature.

short outgrowths arising from the coiled structure (fig. 5, A). There appeared to be actual unions between certain cells of this complex, but this was difficult to determine because of the small diameter of the hyphae. In other cases it was definitely seen that cell fusions occurred, and several types of these are illustrated in figure 5, B. In most cases the hypha "seeking" compatible cells was very slender,

generally less than 1μ in diameter. After fusion with the receptive cell, and the frequent transfer of cell contents, this slender hypha degenerated by gradual autolysis. Cells in which fusion had taken place often formed a group of microconidia (fig. 5, *D*) or repeated the process of growth and further cell fusions. Such hyphal coils, cell fusions, and the production of microconidia in this manner are interesting from the standpoint of their suggestion of possible sexual phenomena. The microconidia bear a striking resemblance to the fertilizing cells found to be necessary for apothecial maturity in

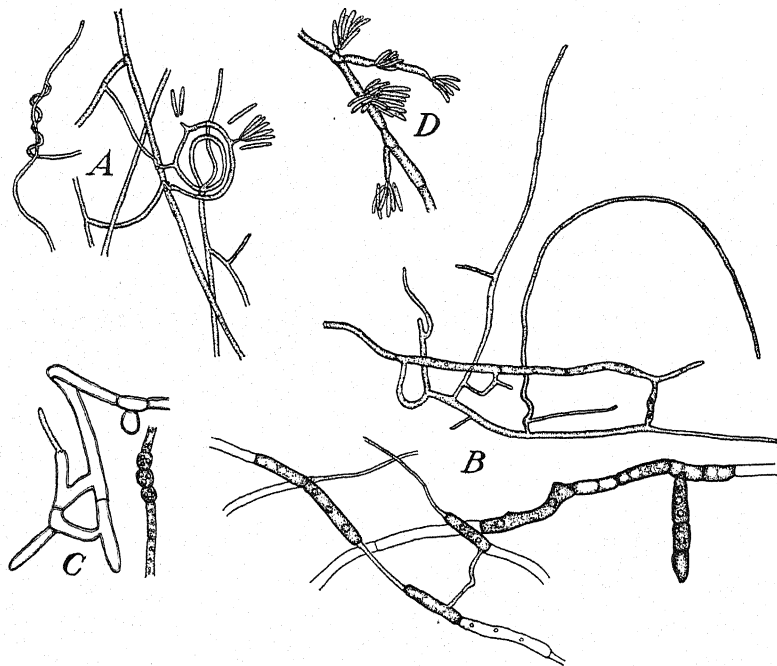


FIGURE 5.—Hyphal coils (*A*), cell fusions (*B*), resting cells (*C*), and microconidia (*D*) produced by both *Neofabraea malicorticis* and *Gloeosporium perennans* in cultures. $\times 312$.

Sclerotinia by Drayton (10) and by Heuberger (22), and in other ascomycetes by Higgins (23).

RESTING HYPHAE AND OTHER ORGANS

Resting hyphae are frequently found in old cultures (fig. 5, *C*) and occasionally in natural cankers; these may serve as an additional means of overwintering or for the survival of these fungi under field conditions. Certain outgrowths commonly appeared in some cultures after they had attained maximum size. At the apexes of these structures (fig. 6) macroconidia were sometimes produced whose presence was indicated macroscopically by the mucoid character of the tips. They resembled acervuli produced in cankers from which the covering bark layer had been removed. In other cases the apexes were pressed firmly against the glass containers and appeared similar to the apothecial fundaments commonly occurring among

certain discomycetes. Since it is known that the apothecia of both the anthracnose and the perennial canker fungi originate within the stromatal mass of old acervuli, these structures may have a connection with the sexual stages in the life cycles of the organisms. The microconidia (or possible spermatia) produced in cultures have been transferred in various combinations to these possible apothecial fundaments without success in producing ascogenous organs. Since in nature these species produce their apothecia in the fall of the second season, the factors necessary for their production by artificial means may be considerably different from the requirements of the species investigated by other workers.

CONCLUSIONS

This general comparative study has failed to distinguish the fungi by common cultural methods. The macroconidia, when produced in cultures by either species under the foregoing conditions, have generally been straight and would be identified as *Gloeosporium perennans*. That typical curved conidia are produced in culture, as reported by Lawrence (27) and by Zeller and Childs (42), is not doubted, but the factors governing conidial curvature under various environmental conditions appear to be very elusive.

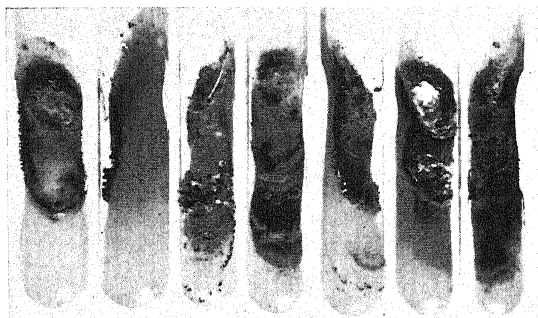


FIGURE 6.—Apotheciumlike structures often found in old cultures.

SPECIAL MEDIA

UTILIZATION OF CARBON AND NITROGEN FROM VARIOUS SOURCES

Eighteen isolations of *Neofabraea malicorticis* and an equal number of *Gloeosporium perennans* were selected for study of their reactions toward carbohydrate and nitrogen from various sources. All cultures were verified at least once by artificial inoculations except A-13, 176, and 177, and these were isolated from cankers typical of the respective diseases. The majority were from single-spore isolations made according to the method recently described by the writer (26). A few cultures from widely separated localities were included for comparative purposes. A detailed record of the cultures is given in table 1.

The use of a synthetic culture medium in liquid form for studies of this kind was found to be impracticable with these fungi. When inoculated directly into the liquid nutrient or upon buoyant materials designed to keep the fungi at the surface, the resulting growth invariably formed in part below the surface. A method of evaluating or comparing such cultures was not devised.

TABLE 1.—Source of cultures used in a comparison of anthracnose and perennial canker fungi

Organism and culture No.	Date isolated	Donor	Locality	Remarks
<i>Neofabraea mali-corticis</i> :				
A-1.....	Sept. 29, 1931	The writer.....	Hood River, Oreg.	From apple rot acervulus.
A-2.....	1930.....	Nellie A. Brown.....	do.....	Canker tissue; Brown's culture No. 4.
A-4.....	Nov. 13, 1928	E. L. Reeves.....	Ferndale, Wash....	Canker tissue; Reeves' culture No. 189.
A-10.....	May 30, 1935	H. R. McLarty.....	Armstrong, B. C....	Canker tissue.
A-11a.....	Nov. 1, 1935	The writer.....	Corvallis, Oreg....	A-11a to A-11i, single conidium isolations from the same collection.
A-11d.....	do.....	do.....	do.....	
A-11h.....	do.....	do.....	do.....	
A-11i.....	do.....	do.....	do.....	
A-12a.....	Nov. 18, 1935	do.....	do.....	A-12a to A-13, single ascospore isolations.
A-12c.....	do.....	do.....	do.....	A-12c and A-12e from same apothecium.
A-12e.....	do.....	do.....	do.....	
A-12h.....	do.....	do.....	do.....	A-12h to A-12k from same apothecium.
A-12i.....	do.....	do.....	do.....	
A-12j.....	do.....	do.....	do.....	
A-12k.....	do.....	do.....	do.....	
A-12n.....	do.....	do.....	do.....	Same collection, but different apothecium.
A-12s.....	do.....	do.....	do.....	Do.
A-13.....	Nov. 15, 1937	do.....	Hood River, Oreg.	From several very large ascospores.
<i>Gloeosporium perennans</i> :				
P-1.....	Feb. 13, 1932	do.....	do.....	4 conidia from apple rot acervulus.
P-2.....	Sept. 29, 1931	do.....	do.....	Reisolation from apple rot acervulus.
P-3.....	Nov. 11, 1931	do.....	do.....	Single conidium from tree canker.
P-4.....	Dec. 22, 1932	E. L. Reeves.....	Wenatchee, Wash....	Canker tissue; Reeves' culture No. 9.
P-8.....	Mar. 21, 1933	R. M. Brien.....	New Zealand.....	From Delicious (?) apple rot.
167a.....	Feb. 15, 1935	The writer.....	Hood River, Oreg....	Single ascospore isolation.
167b.....	do.....	do.....	do.....	Single ascospore from same collection.
173a.....	Dec. 23, 1936	do.....	Mosier, Oreg.....	173a to 173d from same collection. All single ascospore isolations.
173b.....	do.....	do.....	do.....	
173c.....	do.....	do.....	do.....	
173d.....	do.....	do.....	do.....	
174a.....	do.....	do.....	Hood River, Oreg....	Single ascospore isolation.
175a.....	Dec. 24, 1936	do.....	Mosier, Oreg.....	175a to 175d single ascospore isolations from the [same] apothecium.
175b.....	do.....	do.....	do.....	
175c.....	do.....	do.....	do.....	
175d.....	do.....	do.....	do.....	
176.....	Nov. 15, 1937	do.....	Hood River, Oreg....	Multiple ascospore isolation.
177.....	do.....	do.....	Parkdale, Oreg.....	Do.

The fungi produced hyaline, barely visible colonies on plain agar or on synthetic media solidified with agar to which no source of carbon had been added. A slight growth occurred with some strains when the medium was supplied with a source of carbon but no added nitrogen. Apparently sufficient protein material was present in the agar to supply a scant source of nitrogen for some strains of the organisms. However, when a source of nitrogen was used in such media, visible characters were outstanding in contrast to check cultures without such nitrogen. Where any doubt existed as to whether the fungi utilized a certain nitrogenous material, a nutrient medium with the desired source of nitrogen was poured upon cotton in test tubes. In this way, after inoculation a typical mycelial growth was produced which could be directly compared with the check tubes in which visible growth failed to occur.

Since sufficient protein material was present in plain agar to support some growth, a simple nutrient solution was selected to lessen the chance of introducing small amounts of nitrogen from possibly impure chemicals. The medium finally selected was a three-salt solution of half strength devised by Leonian, as reported by Groves (17), with the following composition: Potassium nitrate, 1 gm.; potassium phosphate (primary), 0.5 gm.; magnesium sulfate, 0.25 gm.; ferric chloride, trace; sugar or carbon source, 10 gm.; water, 1,000 cc.

To test the utilization of nitrogen, the potassium nitrate was omitted and 0.5 gm. per liter of the desired source of nitrogen was substituted, except in the case of cysteine monohydrochloride, which was added in the amount of 0.2 gm. per liter. When the medium was used in solid form 1½ percent of agar was introduced. The ability of the different strains of *Neofabraea malicorticis* and of *Gloeosporium perennans* to utilize various carbon sources is shown in table 2.

Little difference was found between the two organisms in their ability to utilize carbon from various sources, either in growth or in other features that might be useful in separating species of fungi. While striking differences between the various strains were often observed, specific distinctions were not apparent. The difference in diastatic activity between the species as reported by Zeller and Childs (42) was the most outstanding. When grown on starchy media, especially corn-meal or oatmeal agar, 9 strains of *Gloeosporium perennans* produced much less clearing of the medium surrounding the growth than did the 2 strains of *Neofabraea malicorticis* that were least active in this respect. Only the perennial canker culture 175b exhibited as great diastatic power as the average of 18 cultures of the anthracnose fungus.

Table 3 shows the comparative growth responses of the fungi when supplied with various sources of nitrogen. As in the previous tests, striking differences often appeared between the strains, but distinction between the two species was not possible.

TABLE 2.—*Ability of Neofabaraa malicorticis and of Gloeosporium perennans to utilize carbon from various sources*
[21-day-old cultures at room temperature on Leonian's medium B]

Organism and culture No.	Growth † on medium B with addition of—															
	No car- bon	Alcohol	Amyg- dalin	Arbu- tin	Dex- trose	Dulci- tol	Galac- tose	Glyc- erin	Inulin	Lac- tose	Levu- lose	Man- nife	Papain	Salicin	Starch	Sucrose
<i>Neofabraea malicorticis</i> :																
A-1.....	Tr	+	+	+ 0	+	+	+	+	Tr	+	+	+	+	+	+	+
A-2.....	Tr	+	+	+ 0	+	+	+	+	Tr	+	+	+	+	+	+	+
A-3.....	Tr	+	+	0	+	+	+	+	Tr	+	+	+	+	+	+	+
A-4.....	Tr	+	+	0	+	+	+	+	Tr	+	+	+	+	+	+	+
A-10.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-11a.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-11d.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-11h.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-11i.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12a.....	Tr	+	+	0	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12c.....	Tr	+	+	0	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12e.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12h.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12i.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12j.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12k.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12n.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-12s.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
A-13.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
<i>Gloeosporium perennans</i> :																
P-1.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
P-2.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
P-3.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
P-4.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
P-8.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
167a.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
167b.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
173a.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
173b.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
173c.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
173d.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
174a.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
175a.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
175b.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
175c.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
175d.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
176.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+
177.....	Tr	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+

TABLE 3.—*Ability of Neofabraea malicorticis and of Gloeosporium perennans to utilize nitrogen from various sources*
[21-day-old cultures at room temperature on Leonian's medium B]

Organism and culture No.	Growth ¹ on medium B with addition of—																		
	No ni- trogen	d-Ala- nine	Ammo- nium chlo- ride	Ammo- nium oxo- late	Ammo- nium sul- late	Ammo- nium tar- trate	Aspar- agine	Cal- cium cyan- amide	Casein	Crea- tine	Cys- teine mono- hydro- chlo- ride	Gela- tin	d-Glu- tamic acid	Glyco- coll	Pep- tone	Potas- sium nitrate	Sodi- um nitrate	Urea	
<i>Neofabraea malicorticis</i> :																			
A-1.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-2.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-3.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-4.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-10.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11a.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11b.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11c.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11d.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11e.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11f.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11g.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11h.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11i.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11j.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11k.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11l.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11m.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11n.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11o.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11p.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11q.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11r.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11s.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11t.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11u.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11v.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11w.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11x.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11y.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-11z.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12a.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12b.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12c.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12d.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12e.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12f.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12g.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12h.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12i.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12j.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12k.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12l.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12m.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12n.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12o.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12p.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12q.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12r.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12s.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12t.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12u.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12v.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12w.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12x.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12y.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-12z.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
A-13.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
<i>Gloeosporium perennans</i> :																			
P-1.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
P-2.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
P-3.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
P-4.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
P-5.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
P-6.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
P-7.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
P-8.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167a.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167b.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167c.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167d.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167e.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167f.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167g.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167h.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167i.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167j.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167k.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167l.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167m.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167n.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167o.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167p.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167q.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167r.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167s.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167t.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167u.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167v.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167w.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167x.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167y.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	
167z.....	Tr	+++	+	+	+	+	+	+	+	Tr	+	+	+	+	+	+	+	+	

¹ 0=no growth; Tr=trade growth; +=20 mm. or less growth in 21 days; ++=more than 20 mm. growth in 21 days.

TOLERANCE FOR TOXIC SUBSTANCES

The greater number of strains used in the carbohydrate- and nitrogen-utilization studies were employed to test the effects of toxic materials incorporated in media as reported by Miller (30) and the writer (25). Tannic acid was incorporated in a medium containing 4 percent of corn meal and 2 percent of agar, while the other materials were used in Leonian's medium B (17), to which 1 percent of dextrose and 1.5 percent of agar were added. When copper sulfate was employed, 3 percent of agar was added to obtain a solid medium. The growth reactions of the fungus strains on these media are shown in table 4.

TABLE 4.—Growth reactions of *Neofabraea malicorticis* and of *Gloeosporium perennans* in media containing toxic materials

[21-day-old cultures at room temperature]

Organism and culture No.	Growth ¹ on media containing indicated concentration of—							
	Tannic acid		Quinone		Malachite green		Copper sulfate	
	0.5 percent	0.9 percent	0.025 percent	0.05 percent	1:400,000	1:200,000	0.05 percent	0.1 percent
<i>Neofabraea malicorticis</i> :								
A-1.....	+++	+	++	+	+	0	+	++
A-2.....	++	0	0	0	+	+	++	+
A-4.....	++	0	0	+	++	0	+	+
A-10.....	+++	+	0	0	+	+	++	++
A-11a.....	+++	+++	++	++	+	+	+++	+++
A-11d.....	+++	+++	0	+	+	+	+++	+++
A-11h.....	+++	+++	0	+	+	+	+++	+++
A-11i.....	+++	+++	0	0	+	+	+++	+++
A-12a.....	+++	+++	+++	++	+	0	++	++
A-12c.....	+++	+++	+++	+++	++	0	+++	++
A-12e.....	+++	+++	0	0	+	+	+++	++
A-12h.....	+++	+++	+++	+++	++	++	+++	++
A-12i.....	+++	+++	++	+++	++	+	+++	++
A-12j.....	+++	++	0	0	+	+	+++	++
A-12k.....	+++	++	+++	0	++	+	+++	++
A-12n.....	++	0	+++	0	+	+	+++	++
A-12s.....	+++	++	0	+++	+++	+	+++	+
A-13.....	+++	++	++	+++	+++	++	++	+
<i>Gloeosporium perennans</i> :								
P-1.....	+++	+++	+++	+++	+++	+++	+	+
P-2.....	+++	+++	0	+++	+++	+++	+	0
P-3.....	+++	+++	+++	+++	+++	+++	++	+
P-4.....	+++	+++	+++	+++	+++	+++	++	+
P-8.....	+++	+++	+++	+++	++	+	+	+
167a.....	+++	+++	+++	+++	++	+++	+	+
167b.....	+++	+++	+++	++	++	+++	++	+
173a.....	+++	+++	+++	0	++	++	++	+
173b.....	+++	+++	+	0	++	+	+	+
173c.....	+++	+++	0	++	+++	++	++	+
173d.....	+++	+++	+++	+++	++	+	+	+
174a.....	+++	+++	+++	+++	++	0	+	+
175a.....	+++	+++	0	0	++	++	0	0
175b.....	+++	+++	+++	++	++	++	++	0
175c.....	+++	+++	++	0	+	+	++	0
175d.....	+++	+++	+++	++	++	++	++	0
176.....	+++	+++	++	++	++	+	+	+
177.....	+++	+++	+++	0	+++	+++	+	+

¹ 0=no growth; +=1 to 10 mm. growth diameter in 21 days; ++=11 to 20 mm. growth diameter in 21 days; +++=more than 20 mm. growth diameter in 21 days.

Growth on media containing quinone was erratic. A depression or a stimulation of the fungi seemed to occur at the time of inoculation but was not consistent when tests were repeated.

Tannic acid inhibited the average growth rate of the strains of *Neofabraea malicorticis* to a greater degree than the strains of *Gloeosporium perennans*. The strengths used brought out no species differences, however, since several of the perennial canker cultures reacted toward tannic acid in a manner similar to the faster growing anthracnose strains.

Malachite green at dilutions of 1 to 400,000 and 1 to 200,000 inhibited the anthracnose cultures to a greater degree than the perennial canker strains. While this difference offered possibilities for separation of the species, several strains of the one were not inhibited to a greater degree than strains of the other.

Copper sulfate at concentrations of 0.05 or 0.1 percent considerably reduced the growth rates of most strains of *Gloeosporium perennans* as compared with those of *Neofabraea malicorticis*. These results are interesting, since copper sprays effectively control the anthracnose fungus in orchards but have little apparent effect upon perennial canker. The differences in mode of infection by the two organisms make it appear probable that the fungicide does not reach the infection court at the proper time in the case of the perennial canker disease.

It has been found impossible to separate the fungi by the tests reported here, but certain responses are indicated that form a basis for future study. By combining the materials showing specific inhibitive tendencies, or by further modification of some of the media, it is possible that a test may be developed suitable for taxonomic use.

VARIATIONS AND SECTORIAL MUTATIONS

GENERAL TYPES

The tendency of some of the cultures to produce color and growth variations was noticed early in the work. There was no difficulty in detecting sectors of the wedge- or fan-shaped type, but mutants formed also as patches, variously colored spots, or irregular sections of distinctive mycelium, which sometimes involved a considerable portion of the growth. Some of the sectors and variations observed in culture are shown in figure 7. Other characters observed may have arisen by mutation. The difficulties involved in being certain that pure lines are isolated have been discussed by Stakman et al. (39). Single ascospore cultures have yielded the greatest number of mutants in these tests, but this may have been accidental. The hyphal coils and cell fusions already mentioned undoubtedly offer an explanation for complex genetic characters within any one culture.

VARIATION OF SINGLE STRAINS IN APPLE BARK

Numerous records on canker sizes accumulated from inoculations during past years revealed confusing variations. Consistent correlations between factors such as inoculation exposures, limb diameters, tree age, and others, could not be made. General conclusions regarding susceptibility of certain types of bark tissue appeared to be warranted, but large variations often occurred in canker sizes in what appeared to be similar tree tissues.

From a knowledge of these variations, a test was designed to study the possibility of fungus variation. Young trees of the Yellow Newtown apple were selected for this purpose. On each tree 10

inoculations with the same isolate were made, starting at the base of a limb and ending near the tip. Only internodal tissue of separate trees, approximately equidistant from the lateral branches, was used, and all other factors were kept as uniform as possible. Inoculations were made December 18, 1934, and records were taken the following summer. The results of this test are given in table 5.

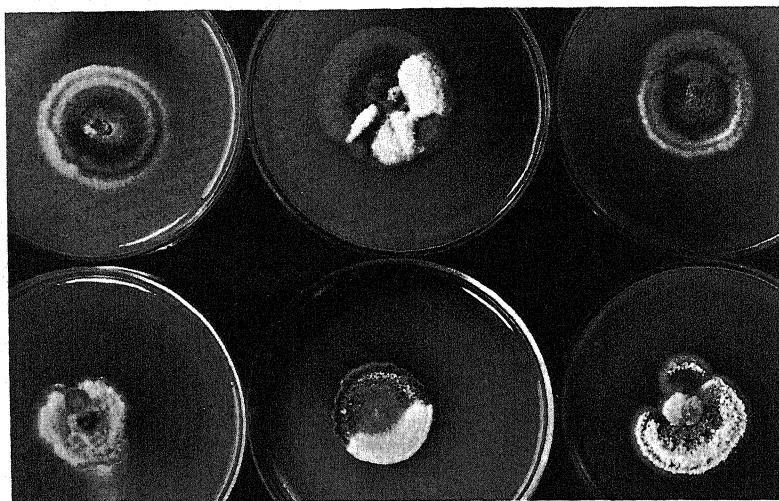


FIGURE 7.—Some types of variation produced by *Neofabraea malicorticis* and *Gloeosporium perennans* in culture.

TABLE 5.—Variation in canker sizes from inoculations with several strains of *Neofabraea malicorticis* and *Gloeosporium perennans* into comparable apple tree tissue

Organism and culture No.	Infection	Canker size			Organism and culture No.	Infection	Canker size		
		Minimum	Maximum	Average			Minimum	Maximum	Average
<i>Neofabraea malicorticis</i> :	Percent	Square millimeters	Square millimeters	Square millimeters	<i>Gloeosporium perennans</i> :	Percent	Square millimeters	Square millimeters	Square millimeters
A-1-----	100	(¹)	800	341	P-1-----	100	150	720	428
A-2-----	30	0	150	15	P-2-----	100	450	600	501
A-3-----	100	160	900	547	P-3-----	100	90	700	314
A-4-----	100	330	1,200	763	P-4-----	100	(¹)	300	144
A-5-----	30	0	270	45	P-5-----	100	450	800	590
A-6-----	0	0	0	0	P-6-----	100	308	1,100	734
A-7-----	10	0	(¹)	(¹)	P-7-----	100	375	900	695
					P-8-----	100	300	1,000	649

¹ Trace.

The data show that considerable variation occurred in canker sizes on what appeared to be comparable bark tissue. That the variation in sizes was not due to differences in host tissue seems reasonable, since the minimum and maximum cankers formed in irregular order. If they were due to host factors, one might reasonably expect a progressive change in sizes from the base to the tips of inoculated limbs.

Previous observations indicated that different strains might vary in their seasonal pathogenicity. A special series of inoculations with strains of known history seemed desirable to test this hypothesis

further. Young trees of the Yellow Newtown apple similar to those used in the preceding inoculation series were used, and the inoculation sites were selected as before. A series of five inoculations with each strain was made September 17, 1936, and another on November 4. Records were taken during the summer of 1937. The results are shown in table 6.

TABLE 6.—*Periodicity of pathogenicity in selected strains of perennial canker and anthracnose fungi inoculated into comparable apple tree tissue*

Organism and culture No.	Inoculated Sept. 17, 1936		Inoculated Nov. 4, 1936		Organism and culture No.	Inoculated Sept. 17, 1936		Inoculated Nov. 4, 1936	
	Infection	Average canker size	Infection	Average canker size		Infection	Average canker size	Infection	Average canker size
<i>Neofabraea malticorticis</i> :		<i>Square millimeters</i>		<i>Square millimeters</i>	<i>Gloeosporium perennans</i> :		<i>Square millimeters</i>		<i>Square millimeters</i>
A-1.....	Percent 20	26	Percent 100	538	P-1.....	Percent 60	156	Percent 100	714
A-2.....	20	34	60	47	P-5.....	100	607	100	1,165
A-10.....	80	586	100	512	167a.....	100	486	100	758
A-11b.....	60	110	100	837	167b.....	100	915	100	584
A-11j.....	0	0	100	470					

These data show that a strain weakly pathogenic at one seasonal period may become strongly pathogenic at a later period. The positive relation between canker size and low temperatures is well established for the perennial canker disease, but it can readily be seen that strains of the organism also vary in their activity at a given period. While the number of inoculations is rather small in the series tabulated, the sites were selected with special care. Numerous unpublished data further substantiate these findings.

COMPARISON OF PARENT AND SECTOR STRAINS UPON INOCULATION INTO APPLE TREES

The time required for a complete life-cycle study of these two canker producing fungi when inoculated into apple trees has limited the accumulation of data on tree-inoculation tests. Sufficiently low temperatures for suitable infections by the perennial canker organism failed to occur during the 2 years that this work was being conducted. However, data regarding the pathogenicity and conidial characters of strains derived from parent cultures have been taken. It seems desirable to present the meager data at this time, since the results have interesting implications.

The sources of the parent cultures were tabulated under a previous heading (table 1). A comparative history of the mutants and their parent cultures is given in table 7.

Each parent and mutant strain was inoculated in parallel series into young trees as the opportunity occurred. The inoculation sites were selected with special care, and all technical features were kept as uniform as possible. A record of the inoculation series is presented in table 8.

The different conidial shapes occurring in this series of inoculations, which are difficult to describe satisfactorily, are shown in figure 8.

TABLE 7.—Characters of parent and sector strains of the anthracnose and perennial canker fungi on media

Original species isolation	Parent or sector	Color ¹ of surface mycelium ²	Color ¹ of back ²	Source of original isolation
Anthracnose.....	{ A-2.....	Deep grayish olive.....	Dragon's-blood red.....	Canker tissue.
	{ A-2a.....	White.....	White.....	Spot mutant.
	{ A-12n.....	Greenish black and grenadine mixture.....	Greenish black and Eugenia red.....	Single ascospore.
Do.....	{ A-12na.....	Greenish black.....	Greenish black.....	Irregular sector.
	{ A-12nb.....	Eugenia red.....	Madder brown.....	Do.
Perennial canker.....	{ 173a.....	Orange cinnamon, begonia rose, and glaucous zone.....	Deeper shade than surface mycelia.....	Single ascospore.
	{ 173s.....	Cinnamon orange.....	Russet.....	Irregular sector.

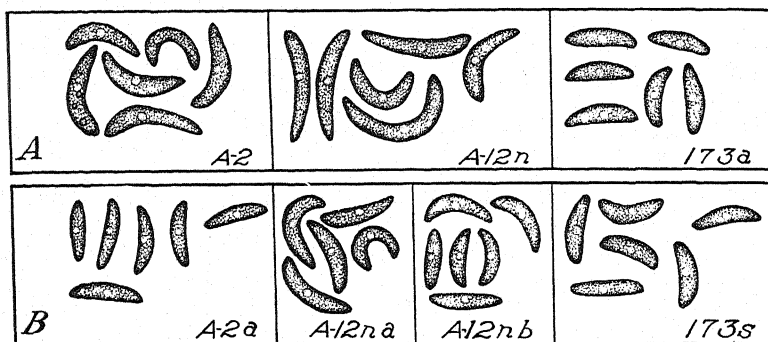
¹ Colors according to Ridgway (37).² 3 weeks' culture on potato 2-percent dextrose agar.FIGURE 8.—Conidia produced from inoculations into apple trees by (A) parent cultures, and (B) sector strains derived from them. $\times 335$

TABLE 8.—Comparison of pathogenicity and conidial characters of parent and sector strains when inoculated into young apple trees

Culture No.	Date inoculated	Inoculations	Infection	Average canker size	Type of conidia produced on trees
	1934	Number	Percent	Square millimeter	
A-2.....	Jan. 3	14	0	—	Perennial canker.
A-2a.....	do.	14	100	282	Anthracnose.
A-2.....	Dec. 18	10	10	15	Perennial canker.
A-2a.....	do.	10	100	744	
	1936				
A-12n.....	Sept. 17	5	100	748	Anthracnose.
A-12na.....	do.	5	80	938	Do.
A-12nb.....	do.	5	20	40	Intermediate.
A-12n.....	Nov. 4	100	100	1,200	Anthracnose.
A-12na.....	do.	5	100	1,052	Do.
A-12nb.....	do.	5	100	1,084	Intermediate.
173a.....	Dec. 29	10	100	658	Perennial canker.
173s.....	do.	10	100	259	Perennial canker (?).

While not definitely established, the results strongly suggest that the perennial canker and anthracnose diseases are caused by strains of a single fungus that may have originated by mutation. More extensive series of inoculations should be made with a greater variety of sector strains. It would be interesting to test further the pathogenicity of the straight-spored strains that have formed directly from anthracnose cultures. Fisher and Reeves (14) have already reported

a parasitic straight-spored strain occurring naturally. It would seem that environmental factors limit the range of the two organisms to distinct climatic districts and that one strain may arise directly from the other. Whether climatic factors cause the fungi to produce mutants that will survive only under these conditions or whether such mutants are able to extend beyond the range of the parent type is not known.

COMPARISON OF APPLE ROTS

Much of the literature on apple anthracnose and perennial canker fruit rots deals with one or the other disease or with methods for their control. The latter phase is beyond the scope of the present study.

Fisher (11) distinguished perennial canker rot from apple anthracnose (1) by slight color differences and by the less zoned appearance, (2) by its infection through lenticels, (3) by its appearance only after long storage, (4) by the production of more copious mycelium in a moist atmosphere, and (5) by its presence in districts where the trees were free from anthracnose cankers. Lawrence (27) obtained curved conidia and microspores in cultures from anthracnose apple rot tissue. In writing of perennial canker conidia, Zeller and Childs (42) state: "They bear a striking contrast to the characteristically curved spores of *Neofabraea malicorticis* from apple fruit." Fisher (12) designated the perennial canker rot as "bull's-eye rot." Several organisms were later found to cause a bull's-eye type of rot (19), and those caused by the perennial canker and anthracnose fungi are not distinguished at market terminals.⁹ Miller (30) compared the temperature relations of the two organisms when inoculated into apple fruits and found them to be very similar. He stated that macroconidia were readily obtained at 10° C., but said nothing of their shapes.

In the present study many series of fruit have been inoculated with known strains of each fungus for the express purpose of observing conidial shapes. Comparative data on the rot characters and conidia on fruit have also been recorded in work on the production of spore material for tree inoculations. In addition, naturally infected fruits have been examined for several years for the purpose of determining the organism concerned.

Numerous examinations of the conidia from rotted fruit grown in the Hood River Valley have shown them to be practically straight, whether developing from known anthracnose or from perennial canker infections. These rots would be classified as perennial canker rot by present standards. Conidia of the decidedly curved type, typical of *Neofabraea malicorticis*, have rarely been encountered in these fruit-rot studies. In the majority of cases the concentric zones of color have predominated in anthracnose rots, but these and other distinguishing characters mentioned by other workers failed to distinguish the species when several strains of each were compared. One has little reason to doubt the statements of Lawrence (27) and of Zeller and Childs (42) that typical curved conidia were produced on apple fruits in their tests. The factors influencing the conidial shapes under different ecological conditions appear to be very elusive and were not discovered in the present studies. Limited trials with

⁹ BRATLEY, C. O. MARKET PATHOLOGY. U. S. Bur. Plant Indus., Plant Dis. Rptr. 15: 38. 1931. [Mimeographed.]

different fruit varieties inoculated at various stages of maturity and with fruit held under different moisture and temperature conditions after inoculation yielded negative results. The moisture and temperature factors should be more fully investigated, however, since they appear to exert a major influence on the fungi in nature.

Ordinarily, sprays applied before harvest reduce the rot appearing in storage¹⁰ (15, 18, 36). This fact suggests that infection of fruit may occur early but remain latent; or possibly the conidia do not germinate until late in the storage life of the fruit. Such infections may complicate results obtained with artificial inoculations, since mixed infections would be possible. A comparative study of the field performance of natural fruit infections by the two fungi has been impossible, for a laboratory means of certainly identifying the two remains unknown. The conidia produced by inoculating apple fruits with known cultures in some of these tests are illustrated in figure 3, A and B.

TAXONOMY OF THE FUNGI

CONIDIAL STAGES OF ANTHRACNOSE AND PERENNIAL CANCKER FUNGI

The acervuli of these fungi are uniformly distributed over the surface of bark cankers. At first they are subepidermal, then erumpent, and under favorable moisture conditions expose a creamy mass of conidia (fig. 9, A and D). The stromata are composed of numerous simple or branched, septate conidiophores (fig. 9, B and E) which arise from elongated series of cells below and bear conidia at their tips. The structural features of the stromatal mass and the conidiophores appear to be identical for both organisms (fig. 9, C and F).

A review of the conidial measurements published by several workers shows little fundamental difference. Conidia of *Neofabraea mali-corticis* were stated by Cordley (?) to measure 6μ by 24μ ; by Peck (34), 4μ by 15μ to 17μ ; and by Lawrence (27), 3.8μ to 5μ by 15μ to 20μ . The conidia of *Gloeosporium perennans*, according to Zeller and Childs (42), measure 4μ to 6μ by 12μ to 20μ . The apple anthracnose conidia have been considered to vary from curved to "hooked," while perennial canker conidia vary from straight to slightly curved. Although this has been found to be generally true, it has been pointed out previously in this paper (p. 638 and fig. 3) that intermediate forms occur which are referable to either species on the basis of these characters.

Examination of the conidia from various localities, on various hosts, in different seasons, have shown them to vary with different environmental conditions. In their typical form apple anthracnose conidia were found to be sickle- to U-shaped, comparatively sharp-pointed and tapered at the end of attachment to the conidiophores, and somewhat broadened below the apex. Typical perennial canker conidia were straight to slightly curved and evenly and abruptly tapered at each end without much variation in width. Measurements of anthracnose conidia under various conditions of development showed them to vary from 3μ to 6μ by 15μ to 35μ . In measuring for length it was possible to find sufficient conidia typical in all respects except for curvature, so that special methods, as used by Blodgett (2) in measuring curved spores, were unnecessary. Perennial canker conidia under similar conditions averaged shorter but ranged from 3μ

¹⁰ See footnote 6.

to 6μ by 12μ to 25μ . Conidia of either species generally showed a spherical, hyaline spot near their centers, but might bear several guttules toward each end, be finely or coarsely granular, or contain one to several oil globules when older. The conidia often became once or twice septate upon germination.

There is, then, a condition where conidia in their typical forms are quite distinct in size, shape, and degree of curvature. In other cases, and especially in the geographical regions where the diseases overlap

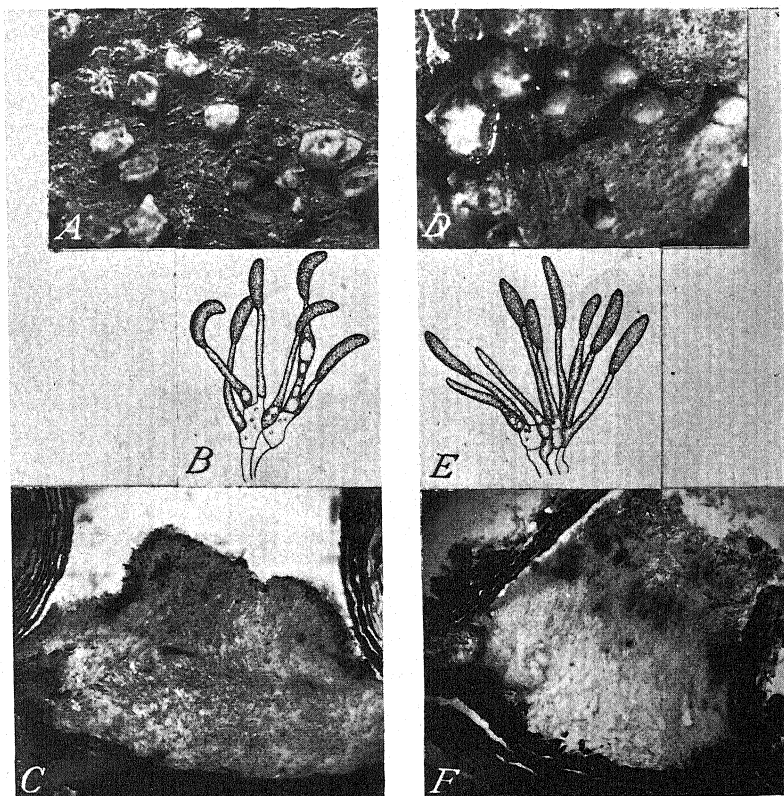


FIGURE 9.—Comparison of the perfect stages of *Neofabraea malicorticis* (A, B, C) and *Gloeosporium perennans* (D, E, F): A and D, Acervuli, \times approximately 6.7; B and E, conidia and conidiophores, \times approximately 355; C and F, cross section of acervuli. \times approximately 67.

in range, the conidia of both species often are of intermediate form, thus making identification from the conidial characters difficult or impossible.

THE APOTHECIAL STAGE OF NEOFABRAEA MALICORTICIS

During the fall of 1935 there was opportunity to study *Neofabraea malicorticis* in its type locality, Corvallis, Oreg. Apothecia were found in abundance, at localities scattered from Corvallis to within a short distance of the Pacific Ocean. Conditions in the shore region appeared to be unfavorable for apothecial development. Moisture

seemed to be the greatest determining factor, and continued humidity after rains was as necessary as periods of rainfall. The apothecial tissues were rather fugitive and often difficult to study even when fresh.

In typical form the ascocarps were found to be from 0.5 to 1 mm. in diameter and of about the same thickness. These became exposed

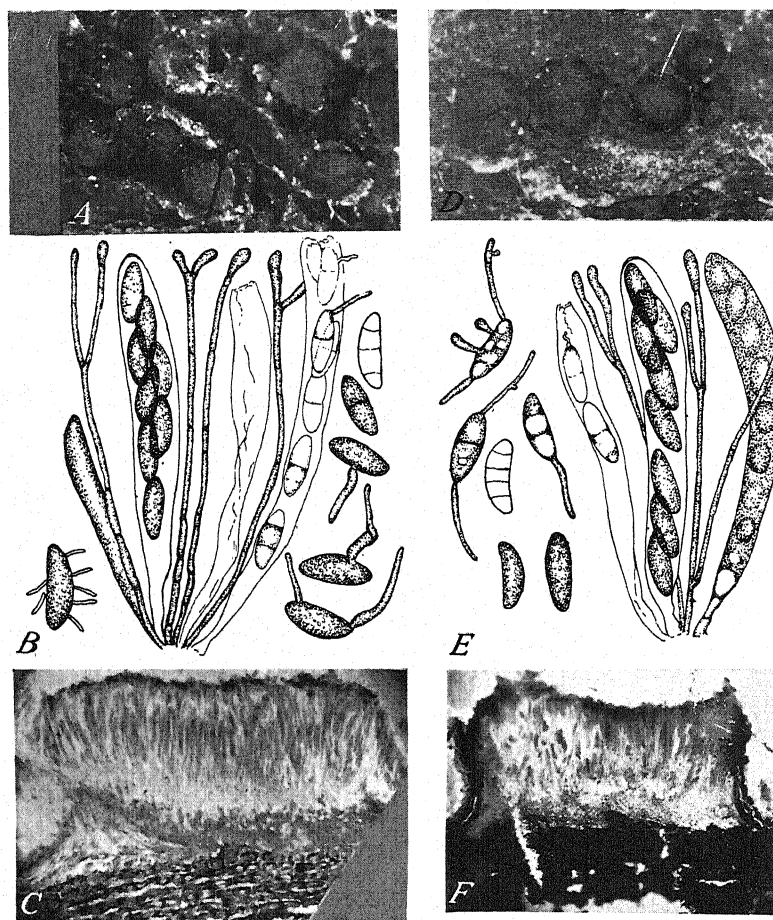


FIGURE 10.—Comparison of the perfect stages of *Neofabraea malicorticis* (A, B, C) and *Gloeosporium perennans* (D, E, F): A and D, Apothecia, \times approximately 6.7; B and E, asci, ascospores, and paraphyses. \times approximately 355; C and F, cross section of apothecia. \times about 67.

at the surface of old conidial stromata as concave or later convex apothecia (fig. 10, A). Occasionally short stipes were present, especially under adverse conditions, but usually the apothecia were sessile. The blackened remains of the conidial stroma often remained clinging to the surface of the apothecium or formed a carbonaceous border around it. Asci could sometimes be found in old conidial stromata before the ascocarps pushed through the stromatic mass. The fruiting body was waxy at maturity, grayish to flesh-colored or

rosy, but gradually deliquesced by an autolytic action into a dark-colored, gelatinous, conglomerate mass. Fresh specimens darkened upon drying. The hypothecium was composed of thin-walled, loosely arranged, colorless cells, appearing like a fine net of irregular mesh (fig. 10, *C*). They merged into smaller and usually more elongated series of cells to form a thin excipular layer that was often bordered by the epidermal cells of host tissue.

Measurements of various collections from the Willamette Valley gave an average ascus measurement of 13.6μ by 102.7μ . The asci were clavate, inoperculate, sometimes slightly broader above the middle, and short-pedicellate at the base. When proper conditions prevailed, the asci elongated, discharged their spores, and then degenerated autolytically. The asci matured progressively so that a new crop of spores was ready to be expelled at each period favorable to spore discharge. Numerous hyaline, simple or branched paraphyses were interspersed among the asci (fig. 10, *B*). They were generally enlarged at the apex to as much as 6μ in diameter. The forms assumed were irregular and often bizarre. Their internal structure was finely granular.

Typical ascospores were hyaline, unicellular, ellipsoid, and often had one side somewhat flattened. The contents of the spores were either coarsely granular or contained many tiny oil globules. Germ tubes pushed out from any point in the spore wall during germination. While generally disposed in a single series, the ascospores were often partly two-seriate or somewhat crowded when about to be discharged. Measurements of 373 ascospores gave an average width of 6.4μ and a length of 19.3μ . In a degenerate state or under conditions unfavorable to spore expulsion, ascospores were commonly found to germinate within the ascus (fig. 10, *B*) and secondary conidia were often produced from them as described by Jackson (24). Under such unfavorable conditions, or with age, the ascospores frequently became one- to four-septate, and light amber or brownish in color. It was definitely established, however, that septate and colored spores are products of age or unfavorable environment and are not to be considered typical of the species. The entire ascus tissue became darker with age.

From this restudy of the species in the type locality and from data given in detail later in the paper, the original description is amended as follows:

Neofabraea malicorticis (Cordley) Jackson, 1913, *Oreg. Bien. Crop Pest and Hort. Rpt.* 1911-12: 178-197.

Synonymy: *Gloeosporium malicorticis* Cordley, 1900. *Bot. Gaz.* 30: 57. Also: 1900, *Oreg. Agr. Expt. Sta. Bull.* 60: 4-7; and 1900, *Oreg. State Bd. Hort. Bien. Rpt.* 6: 405-409.

Macrophoma curvispora Peck, 1900, *Torrey Bot. Club Bul.* 57: 21.
Myzospodium curvisporum (Peck) Sacc., 1907, *Wash. Agr. Expt. Sta. Bul.* 83: 32-33.

Pezizula malicorticis (Jackson) Nannfeldt, 1932, *Nova Acta R. Soc. Sci. Upsala.*, (4) 8: 91, 92.

Cryptosporiopsis malicorticis (Cordley) Nannfeldt, 1932, *Nova Acta R. Soc. Sci. Upsala.*, (4) 8: 91.

Apothecia sessile or occasionally short-stipitate, at first concave, later flat or convex, 0.5 to 1 mm. in diameter and about the same thickness, surrounded at the edge by the blackened remains of enveloping layers, grayish to flesh-colored or rosy and waxy when fresh, becoming darker upon drying or with age, and finally gelatinizing by autolysis; hypothecium composed of thin-walled, loosely arranged, colorless, irregular cells that merge into smaller and usually more elongated series extending to form a thin excipular layer, often bordered by epidermal

cells of the host; asci clavate, inoperculate, usually slightly broader above the middle and short-pedicellate at the base, 10μ to 20μ by 75μ to 150μ (mean 13.6μ by 102.7μ); paraphyses granulose, filiform, septate, simple or branched, irregularly enlarged up to 6μ at the apex, hyaline; ascospores unicellular, ellipsoid, often somewhat flattened on one side, coarsely granular or finely guttulate, hyaline or becoming amber or brownish with age, 5μ to 9μ by 12.5μ to 26μ (mean 6.4μ by 19.3μ , mode 6μ by 20μ), often becoming one- to four-septate upon germination, one- or partly two-seriate.

Acervuli formed in cankers the first season, at first subepidermal, then erumpent, 0.2 to 1 mm. in diameter, exposing a creamy mass of conidia borne on simple or branched hyaline conidiophores; macroconidia unicellular, sometimes one-septate upon germination, sickle- to U-shaped, occasionally straight, coarsely granular to finely guttulate or with one to several oil globules at maturity, hyaline, 3μ to 6μ by 15μ to 35μ .

Developing in the fall and winter on dead bark of *Malus sylvestris* Mill., *M. rivularis* Roemer, or *Pyrus communis* L., in cankers formed by the conidial stage, *Gloeosporium malicorticis*, the previous season.

Type locality: Corvallis, Oreg.

DISCOVERY OF APOTHECIA OF GLOEOSPORIUM PERENNANS

During the course of studies with perennial canker in 1928 and 1929, J. S. Cooley, of this Division, observed an ascomycetous fungus associated with the lesions of perennial apple cankers. He studied the essential features from the meager material he was able to collect and prepared illustrations. The detailed features were very similar to those given for *Neofabraea malicorticis*, but he was inclined provisionally to give the fungus the varietal name *perennans*.¹¹ The description and illustrations accompanying his data clearly indicate that he had discovered the apothecial stage but that his material had developed under adverse conditions or was overmature.

A constant search by the writer was rewarded by finding apothecia resulting from artificial inoculations made on apple trees in 1932 with a known strain of *Gloeosporium perennans*. Apothecia were also discovered for the first time on pear trees in cankers resulting from artificial inoculations. These apothecia appeared in the early winter of 1935, at which time weather conditions were suitable for their formation. A further search that year yielded additional material from areas in which anthracnose cankers were absent. The following season proved to be more suitable for apothecial formation at Hood River, Oreg., and several collections of the material were made. The presence of apothecia in cankers of known origin and the collection of material in areas known to be free from anthracnose made identification relatively certain. Identification was confirmed by the frequent presence of typical conidia in the same stromata.

COMPARISON OF APOTHECIAL STAGES

A superficial examination revealed little difference between the apothecia of the two fungi (fig. 10, A and D). In their natural state apothecia of *Neofabraea malicorticis* were generally found at the center of cankered tissue or might be evenly distributed over the surface. Apothecia of the perennial canker fungus were most frequently found on the thin pellicles of bark being sloughed from the original aphid-injured and infested callus tissue common to this disease. In either case, since their forms and appearances resembled quite closely those of matured acervuli, they might easily be overlooked. A knowledge

¹¹ COOLEY, J. S. ASCOSPORE STAGE OF GLOEOSPORIUM PERENNANS ZELLER AND CHILDS. [Unpublished data.]

of the manner in which natural infections occur and of the appearance of cankers in their various stages is most helpful in an evaluation of specific characters.

The largest apothecial collection of *Gloeosporium perennans* was made near Mosier, Oreg., from dead apple trees that had been uprooted at least 2 years before. It was interesting to observe that ascocarps often developed in this case directly on the wood from which bark cankers had sloughed and would hardly have been suspected of being related to the perennial canker disease without the presence of adjacent intact cankers. The ascocarps under this condition were more gregarious than usual, even becoming effuse, and more irregular in shape, several occasionally coalescing to form an undulate hymenium. Apothecia of *G. perennans* developing from artificial inoculations and those forming naturally on bark tissue corresponded in size and shape to those of *Neofabraea malicorticis*. A comparison of the apothecial colors showed a tendency for translucent gray and flesh tints to predominate in those produced in perennial cankers in the dry sections east of the mountain range, while pinkish tints predominated in those from anthracnose cankers in the wetter Willamette Valley. Color differences were not consistent and formed no basis for separating the organisms. In the latter region the apothecia remained fresh longer because of moisture conditions, while in the former they were found only during rainy periods and rapidly shrank into the stromatic mass upon the cessation of the rain. Such differences in weather conditions might easily be responsible for slight variations in color.

Comparative measurements made from apothecia collected during several years are summarized in table 9, and the structural features are illustrated in figure 10.

TABLE 9.—Measurements of apothecial material of *Gloeosporium perennans* and of *Neofabraea malicorticis*

Organs measured and character	<i>Gloeosporium perennans</i>	<i>Neofabraea malicorticis</i>
Apothecia, diameter.....millimeters	0.5-1.	0.5-1.
Asci:		
Typical size..... μ	14.2 \times 102.	15 \times 110.
Range..... μ	8.5-19.8 \times 50-142.	10-20 \times 75-150.
Mean average..... μ	12.04 \times 98.1.	13.6 \times 102.7.
Ascospores:		
Number measured.....	465.	373.
Typical size..... μ	6.2 \times 19.8.	6 \times 20.
Range..... μ	4.2-8.5 \times 11.5-22.7.	5-9 \times 12.5-26.
Mode..... μ	5.7 \times 17.5.	6 \times 20.
Mean..... μ	6 \times 17.7.	6.3 \times 19.6.
0-septate.....percent	65.8.	84.7.
1-septate.....do	6.0.	4.1.
2-septate.....do	7.0.	4.6.
3-septate.....do	20.0.	6.0.
4-septate.....do	1.0.	4.
5-septate.....do	2.	2.
Paraphyses at apex, diameter..... μ	Up to 7.	Up to 6.

¹ Sometimes coalescing to form undulate hymenia.

The variation that may occur in ascospore measurements made by different workers, from different hosts, or during different seasons is shown in table 10.

TABLE 10.—Comparison of measurements of ascospores of *Neofabraea malicorticis* and *Gloeosporium perennans*

Organism, worker, host, ¹ and locality	Ascospores measured	Mean length	Mean width
<i>Neofabraea malicorticis</i> :	Number	μ	μ
All collections by the writer	373	19.6	6.3
Willamette Valley, Oreg.	323	19.3	6.4
Hood River, Oreg.	50	21.5	5.8
Jackson's description (24)	(?)	16-19	5-7
<i>Gloeosporium perennans</i> :			
All collections by the writer	465	17.7	6.0
Hood River, Oreg.	280	17.6	5.7
Mosier, Oreg.	100	17.6	6.2
Parkdale, Oreg.	50	17.0	5.3
Anjou pear, Hood River, Oreg.	25	17.1	7.3
Cooley's measurements ²	(?)	15.7	5.3

¹ Apple unless otherwise stated.² See footnote 11.

An examination of tables 9 and 10 shows the close similarity between the ascogenous stages of *Neofabraea malicorticis* and *Gloeosporium perennans*. Inasmuch as the apothecia developed under different conditions of moisture and during different seasons, such variations in measurements as are shown might be expected. A separation of these forms as species based wholly on morphological or physiological grounds appears unwarranted. Because of the practical aspects of disease control, however, it seems desirable to consider them as distinct species, for the diseases caused by them must be handled as distinct diseases in the orchard. A further study of the factors influencing such characters as conidial curvature, origin of new strains, and conditions associated with their parasitic or saprophytic tendencies may eventually justify a closer combination. Cytological studies of these fungi might be very helpful in this respect.

It seems advisable at the present time to retain the specific rank of the perennial canker fungus. The specific name *perennans* is here proposed for the apothecial stage of *Gloeosporium perennans*, and the fungus is described as follows:

***Neofabraea perennans* (Zeller and Childs) comb. nov.**

Synonymy: *Gloeosporium perennans* Zeller and Childs, 1925, Oreg. Agr. Expt. Sta. Bul. 217.

Differing from *Neofabraea malicorticis* (Cordley) Jackson in having slightly smaller asci and ascospores and in having mostly straight or slightly curved conidia. Asci 8.5μ to 19.8μ by 50μ to 142μ (mean 12.64μ by 98.1μ); ascospores 4.2μ to 8.5μ by 11.5μ to 22.7μ (mean 6μ by 17.7μ , mode 5.7μ by 17.5μ); macroconidia straight to slightly curved, occasionally strongly curved, 3μ to 6μ by 12μ to 25μ . Developing in the fall and winter on dead bark or wood of *Malus sylvestris* Mill. or *Pyrus communis* L. in cankers formed by the conidial stage, *Gloeosporium perennans*, the previous season.

Type locality: Hood River, Oreg.

A *Neofabraea malicorticis* differt ascis et ascosporis paulum minoribus et conidiis plerumque rectis vel leniter curvulis. Ascis 8.5μ – 19.8μ latis, 50μ – 142μ longis; ascosporis 4.2μ – 8.5μ latis, 11.5μ – 22.7μ longis; macroconidiis rectis usque leniter curvulis, interdum valde curvatis, 3μ – 6μ latis, 12μ – 25μ longis. Autumno et hieme in cortice et ligno emortuo *Malus sylvestris* et *Pyrus communis* in cancris a statu conidico. *Gloeosporio perennanti*, in tempore priore formati, Hood River, Oreg.¹²

Under natural conditions the disease caused by this fungus is generally distinguished from apple anthracnose by its failure to be

¹² The writer is indebted to Edith K. Cash, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture, for the Latin description.

controlled by copper sprays and its habit of infecting injured bark tissue, especially galls produced by the woolly apple aphid and ruptured at low temperatures. Typical conidia may be distinguished by shape.

The type specimen has been deposited in the herbarium of the Department of Botany, Oregon State College, Corvallis, Oreg., and typical material in the mycological collections of the Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

SUMMARY

Apple anthracnose, caused by *Neofabraea malicorticis*, and perennial canker, due to *Gloeosporium perennans* infections, are very closely related. Natural cankers are quite distinct, but the organisms could not be distinguished by means of morphological, physiological, or mycological studies.

Anthracnose occurs mainly west of the Cascade Range, and perennial canker east of that range. Both occur in certain overlapping districts, and here the causal fungi tend to be intermediate in type. Identification from the conidial characters is difficult under such conditions.

The fungi infected the same host plants when artificially inoculated. Specific separation was impossible from the characters exhibited in laboratory cultural tests on various media. Strains of the same form showed considerable difference in reaction in laboratory cultures.

Copper fungicides protect against anthracnose infections in the orchard but fail to affect perennial canker infections materially. On the other hand, the perennial canker fungus was inhibited to a greater degree than the anthracnose fungus when grown on media containing copper sulfate.

Both fungi frequently produced sectors in culture. Conidia typical of the perennial canker fungus and also intermediate forms were produced by inoculation of mutants derived from anthracnose cultures. These results suggest that the two fungi may have arisen from a single species by mutation.

A distinction between apple rots produced from inoculations with the two fungi could not be made. Conidia produced by either fungus on apple fruits were of the perennial canker type.

The apothecial stage of *Gloeosporium perennans* is described and compared with *Neofabraea malicorticis*. A slight average difference in ascospore dimensions was found, but specimens that developed under favorable environmental conditions were indistinguishable from apothecia of the anthracnose fungus. The perennial canker fungus is retained as a distinct species because of its pathological differences and is named *Neofabraea perennans*.

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FURTHER STUDIES ON ROOT CHARACTERISTICS OF WINTER WHEAT IN RELATION TO WINTER INJURY¹

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INTRODUCTION

Previous studies on the roots of winter wheats have been reported,² the object of which was to find some characteristics associated with ability to survive the winter season, especially where damage from heaving was an important consideration. The experiments, although rather extensive, were of a preliminary nature. The main factors investigated were size of root, tensile strength, and stretching ability. Tests were run on the nine series given below. In all cases varieties were chosen from among those included in the Eastern Uniform Winterhardiness Nursery.

(1) The H series—preliminary studies on eight varieties.

(2) The S and 2S series—15 varieties planted in the spring, and tests run at two stages of growth (the second set incomplete). The object was to provide growing conditions widely different from the H series.

(3) The W and 2W series—fall and spring determinations on 15 varieties grown at Wooster, Ohio.

(4) The C and 2C series—fall and spring determinations on the same 15 varieties grown at Ithaca, N. Y.

(5) The pot series—grown in the greenhouse at Cornell University.

(6) The L series—a very brief study of two varieties, each grown at four fertility levels.

In a summary of the work, which extended over three seasons, the following statement was made:³

* * * the studies reported here indicate clearly that certain root measurements vary markedly with variety and with the resistance of the variety to cold and to heaving injury. Observations combined with the results of the experiments further indicate that no one characteristic of the roots alone is an entirely satisfactory measure of ability to resist heaving and that a number of attributes not studied as yet may have considerable significance in determining behavior.

The present paper is devoted to the further study of this problem, and reports a number of experiments supplementing those already presented.

Extensibility of roots was dropped from consideration, partly because this characteristic seemed the least important in the development of an empirical technique, and partly because difficulty was encountered in obtaining reliable data. Emphasis was put on tensile strength as affording the most promising avenue of attack. Measurement of stele diameter was made in all the new series of experiments, since it was clear that the cortex played little or not part in root strength.

¹ Received for publication February 28, 1939. Contribution from the Department of Agronomy, Ohio Agricultural Experiment Station.

² LAMB, C. A. TENSILE STRENGTH, EXTENSIBILITY, AND OTHER CHARACTERISTICS OF WHEAT ROOTS IN RELATION TO WINTER INJURY. Ohio Agr. Expt. Sta. Bul. 568, 44 pp., illus. 1936.

³ See p. 43 of reference cited in footnote 2.

Five series of experiments are reported here, all from plots grown at Wooster, Ohio, in the seasons 1935-36 and 1936-37. The principal points under consideration were:

- (1) Further study to find the season of the year at which samples should be taken and determinations made.
- (2) A critical consideration of the value of stele measurements.
- (3) The influence of level of fertility at which the crop is grown.

The technique of sampling and the details of methods were exactly as described in the earlier publication, except that data on extensibility were omitted and those on stele diameter added. With good light, the outline of the stele was discernible under the low power of the microscope, and was measured with the micrometer eyepiece. Briefly, well-developed permanent roots were selected, diameter of the whole root and vascular stele recorded, and the breaking tension determined with the special apparatus developed.

Data tabulated or calculated for each individual root were: (1) Diameter of root in millimeters; (2) diameter of stele in millimeters; (3) cross section of root in square millimeters; (4) cross section of stele in square millimeters; (5) breaking tension in grams; (6) breaking tension in grams per millimeter of root diameter; (7) breaking tension in grams per millimeter of stele diameter; (8) breaking tension in grams per square millimeter of root cross section; (9) breaking tension in grams per square millimeter of stele cross section and (10) thickness of cortex.

Means with standard errors for each of these, and correlations between breaking tension and each of the root measurements were calculated.

PRESENTATION OF DATA

Six varieties of wheat (*Triticum aestivum* L.) were chosen for planting in the fall of 1935. Minhardi, Fulhio, and Gladden were included to provide a wide range of root size and strength, together with Thorne, Baldrock, and Purdue No. 1, three new and promising sorts developed in Ohio, Michigan, and Indiana, respectively. One series of determinations was run on these in the fall (3W series) and a second in the early spring (4W series). The results are given in table 1.

TABLE 1.—*Root, stele, and cortex measurements, and breaking-tension data from 6 varieties of wheat in fall and spring studies*[*n*=200 in all cases]

Characteristic	Series ¹	Minhardi	Fulhio	Gladden	Thorne	Baldrock	Purdue No. 1
Diameter of root	3W	0.545±0.004	0.629±0.005	0.635±0.006	0.653±0.005	0.634±0.005	0.607±0.005
millimeters	4W	0.417±0.004	0.509±0.005	0.529±0.005	0.506±0.005	0.498±0.004	0.468±0.004
Diameter of stele do	3W	0.408±0.004	0.482±0.005	0.492±0.005	0.487±0.005	0.467±0.003	0.451±0.004
Cross section of root	3W	0.237±0.004	0.313±0.005	0.320±0.006	0.338±0.006	0.315±0.005	0.290±0.005
square millimeters	4W	0.140±0.003	0.210±0.004	0.223±0.004	0.207±0.004	0.200±0.003	0.177±0.003
Cross section of stele	3W	0.135±0.002	0.189±0.004	0.197±0.004	0.193±0.004	0.176±0.003	0.165±0.003
square millimeters	4W	0.084±0.003	0.126±0.004	0.131±0.004	0.127±0.004	0.119±0.003	0.112±0.003
Breaking tension, grams	3W	240.8±4.74	396.2±8.13	438.8±6.93	394.5±7.25	460.8±7.88	323.5±5.90
Per millimeter of root diameter	4W	234.0±4.03	417.8±6.26	492.0±6.12	422.5±6.12	486.3±6.68	344.3±4.53
Breaking tension, grams	3W	439.5±8.1	627.0±11.5	690.5±10.6	597.3±9.4	722.5±10.5	535.0±8.91
Per millimeter of stele diameter	4W	567.5±10.2	826.8±14.1	939.5±14.0	832.8±12.7	980.0±13.7	732.8±9.30
Per square millimeter of root cross section	3W	593±10.4	816±14.3	891.0±14.3	803.3±12.2	979.8±14.1	715.0±11.7
grams	4W	1,040±20	1,289±25	1,422±28	1,185±20	1,474±22	1,145±21
Per square millimeter of stele cross section	3W	1,768±40	2,129±47	2,294±37	2,128±42	2,535±46	2,018±35
grams	4W	1,888±39	2,173±41	2,350±49	2,136±42	2,690±46	2,039±42
Thickness of cortex	3W	0.073±0.001	0.075±0.001	0.072±0.001	0.084±0.001	0.084±0.001	0.078±0.001
Correlations:							
Breaking tension and root diameter	3W	+0.351	+0.439	+0.283	+0.565	+0.550	+0.366
Breaking tension stele diameter	4W	+0.112	+0.022	+0.104	+0.189	+0.259	+0.394
Breaking tension root cross section	3W	+0.370	+0.482	+0.277	+0.570	+0.717	+0.400
Breaking tension stele cross section	4W	+0.340	+0.416	+0.283	+0.567	+0.531	+0.361
Breaking tension and stele cross section	4W	+0.089	+0.039	+0.091	+0.143	+0.247	+0.378
Breaking tension and root diameter	3W	+0.364	+0.479	+0.274	+0.552	+0.509	+0.402

¹ 3W=fall; 4W=spring.*r*>0.138 significant (*P*<0.05); *r*>0.181 highly significant (*P*<0.01).

At the Ohio Agricultural Experiment Station in 1928, a 3-year rotation of wheat, oats, and corn was set up at four fertility levels to levels to study the effect of soil productivity on different varieties of these crops. The results have been published elsewhere.⁴ In the fall of 1935, eight varieties of wheat were grown on these plots. One replication was chosen, and samples were taken in the early winter from each variety at each fertility level for root studies. Data are presented in table 2.

Information was wanted on a number of new lines, and a special planting, which included 25 strains and varieties, was made in the fall of 1936. These were planted with the hand drill in the wheat nursery at 7-inch spacing (5W series). Ten of these varieties were repeated, and the seeds dropped by hand approximately three-fourth of an inch apart (6W series) to see whether greater uniformity of plants and a consequent reduction in variability might be obtained if seed were space-planted in the row. Data for these two series are given in table 3.

⁴ STRINGFIELD, G. H., and SALTER, ROBERT M. DIFFERENTIAL RESPONSE OF CORN VARIETIES TO FERTILITY LEVELS AND TO SEASONS. *Jour. Agr. Research* 49: 991-1000, illus. 1934.

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— and SALTER, ROBERT M. RESPONSE OF OAT VARIETIES TO DIFFERENT FERTILITY LEVELS. *Jour. Agr. Research* 55: 785-793, illus. 1937.

TABLE 2.—Root and stele measurements and breaking-tension data for eight varieties of winter wheat grown at four fertility levels (2L series)

Characteristic	Fertility level ¹	Minhardt	Fulbio	Gladden	Trumbull	Thorne	Test No. 1029	Dawson	Michkof
Diameter of root.....millimeters	A	0.357±0.004	0.377±0.007	0.409±0.008	0.393±0.007	0.349±0.006	0.360±0.006	0.395±0.007	0.381±0.006
	B	0.374±0.005	0.427±0.007	0.486±0.006	0.496±0.008	0.490±0.006	0.459±0.007	0.450±0.006	0.406±0.006
	C	0.389±0.007	0.524±0.007	0.553±0.007	0.532±0.008	0.531±0.008	0.492±0.006	0.408±0.006	0.438±0.006
	D	0.289±0.008	0.550±0.007	0.572±0.007	0.548±0.008	0.584±0.006	0.523±0.006	0.527±0.007	0.477±0.007
Diameter of stele.....do	A	0.213±0.008	0.211±0.005	0.232±0.005	0.249±0.004	0.196±0.003	0.212±0.003	0.218±0.003	0.215±0.003
	B	0.216±0.004	0.244±0.004	0.304±0.006	0.340±0.006	0.277±0.003	0.255±0.004	0.259±0.004	0.220±0.003
	C	0.218±0.004	0.327±0.006	0.324±0.006	0.355±0.007	0.221±0.003	0.285±0.004	0.267±0.004	0.242±0.004
	D	0.200±0.007	0.319±0.004	0.330±0.004	0.341±0.007	0.284±0.004	0.303±0.004	0.307±0.003	0.268±0.005
Cross section of root.....square millimeters	A	0.100±0.002	0.118±0.004	0.138±0.005	0.127±0.004	0.101±0.003	0.107±0.004	0.128±0.004	0.120±0.004
	B	0.114±0.003	0.153±0.005	0.192±0.006	0.199±0.006	0.125±0.004	0.171±0.005	0.171±0.004	0.135±0.004
	C	0.131±0.005	0.220±0.005	0.246±0.007	0.229±0.007	0.181±0.006	0.194±0.004	0.178±0.004	0.155±0.004
	D	0.167±0.006	0.241±0.006	0.258±0.006	0.242±0.007	0.180±0.005	0.218±0.005	0.224±0.006	0.184±0.006
Cross section of steel.....do	A	0.034±0.002	0.037±0.002	0.043±0.002	0.052±0.002	0.028±0.001	0.035±0.002	0.037±0.002	0.037±0.002
	B	0.038±0.002	0.051±0.002	0.078±0.003	0.097±0.004	0.040±0.002	0.057±0.002	0.058±0.002	0.037±0.002
	C	0.039±0.002	0.088±0.002	0.087±0.003	0.105±0.004	0.062±0.002	0.071±0.002	0.062±0.002	0.051±0.002
	D	0.058±0.003	0.087±0.002	0.090±0.002	0.097±0.004	0.068±0.002	0.077±0.002	0.077±0.002	0.060±0.002
Breaking tension.....grams	A	247.0±5.95	267.0±6.00	282.5±6.85	273.5±6.40	276.5±6.25	246.0±5.40	280.5±6.05	265.5±4.99
	B	221.5±5.40	327.0±7.35	386.0±9.25	310.0±8.40	297.0±6.45	282.0±7.85	353.5±7.80	283.5±5.50
	C	221.0±5.05	450.5±10.15	429.5±8.95	339.0±10.05	318.0±7.00	348.0±8.50	341.0±7.40	306.0±5.85
	D	237.5±6.45	496.5±8.55	497.5±6.85	409.5±9.15	345.0±8.25	386.0±8.30	434.5±9.00	374.5±9.45
Breaking tension: Per millimeter of root diameter.....do	A	691.0±16.6	722.5±18.7	698.5±17.4	706.5±19.1	795.5±22.7	704.5±18.6	750.0±19.9	708.5±16.4
	B	608.0±16.7	764.0±17.4	794.0±16.9	632.0±16.9	771.0±19.6	621.0±16.2	766.5±17.3	709.5±15.3
	C	574.0±17.1	838.0±17.8	779.5±17.2	634.0±17.1	675.5±16.8	712.0±18.6	718.5±14.5	694.5±14.6
	D	526.5±13.4	904.0±17.2	869.5±14.6	751.0±16.9	704.0±16.1	741.0±14.7	830.0±19.2	775.0±16.6
Per millimeter of stele diameter.....do	A	1.133±0.26	1.339±0.41	1.219±0.30	1.098±0.28	1.414±1.6	1.179±0.30	1.349±0.35	1.246±0.36
	B	1.053±0.20	1.335±0.27	1.272±0.28	1.098±0.25	1.336±3.5	1.092±0.28	1.353±0.31	1.292±0.26
	C	1.033±0.31	1.397±0.33	1.338±0.30	988±27.5	1.165±27.1	1.208±0.30	1.251±22.8	1.252±25.0
	D	937±25.8	1.548±27.8	1.515±26.0	1.228±31.5	1.187±26.2	1.270±25.5	1.446±31.3	1.381±29.3
Per square millimeter of root cross section.....do	A	2.541±7.7	2.599±10.5	2.269±8.6	2.341±8.9	3.030±12.5	2.617±10.3	2.532±10.1	2.463±9.2
	B	2.168±8.0	2.348±8.1	2.105±6.4	1.670±5.5	2.604±7.1	1.752±6.9	2.162±6.5	2.272±7.4
	C	1.955±8.7	2.145±5.6	1.845±5.5	1.549±4.8	1.876±6.1	1.841±5.6	1.954±4.6	2.066±6.0
	D	1.542±6.5	2.009±4.9	1.600±4.9	1.796±5.4	1.869±4.8	1.829±4.6	2.052±6.1	2.133±5.8
Per square millimeter of stele cross section.....do	A	6.812±20.4	9.230±58.7	6.976±28.0	5.784±23.4	9.364±44.2	7.230±27.8	8.338±40.4	7.501±22.5
	B	6.334±25.5	6.980±20.5	5.556±19.9	5.598±12.7	7.978±31.6	5.430±18.2	6.648±19.7	7.600±23.6
	C	6.644±19.0	5.418±17.6	5.418±17.6	3.634±14.0	5.600±18.5	5.348±15.7	5.806±14.5	6.680±22.4
	D	4.976±20.8	5.924±14.7	5.924±14.7	4.796±17.9	5.252±12.9	5.304±12.9	6.052±15.5	6.640±20.7

[n = 100 in all cases]

Correlations:											
Breaking tension and root diameter	A										
	B										
	C										
	D										
Breaking tension and stele diameter	A										
	B										
	C										
	D										
Breaking tension and root cross section	A										
	B										
	C										
	D										
Breaking tension and stele cross section	A										
	B										
	C										
	D										

1 Level A received no added plant nutrient in any form and was the poorest; levels B, C, and D provided increasingly productive conditions by the addition of 1, 2, and 4 increments of fertilizer materials. For further information, see the two references by Lamb and Salter, cited in footnote 4, p. 669.

$r > 0.195$ significant ($P < 0.05$); $r > 0.264$ highly significant ($P < 0.01$).

-0.053	+0.213	+0.355	+0.207	0.000	+0.078	+0.057	+0.090
-0.101	+0.435	+0.389	+0.279	+0.138	+0.361	+0.213	+0.120
+0.032	+0.354	+0.229	+0.388	+0.140	+0.170	+0.353	+0.191
+0.346	+0.137	+0.113	+0.321	+0.359	+0.321	+0.043	+0.377
+0.136	+0.220	+0.379	+0.236	+0.008	+0.094	+0.069	+0.193
+0.020	+0.514	+0.407	+0.222	+0.075	+0.343	+0.148	+0.210
+0.338	+0.284	+0.245	+0.331	+0.233	+0.129	+0.458	+0.331
-0.043	+0.219	+0.148	+0.257	+0.393	+0.133	+0.133	+0.480
-0.172	+0.208	+0.367	+0.253	-0.011	+0.125	-0.020	+0.013
+0.038	+0.409	+0.417	+0.272	+0.110	+0.310	+0.183	+0.113
+0.308	+0.168	+0.211	+0.393	+0.146	+0.193	+0.368	+0.162
+0.059	+0.438	+0.081	+0.349	+0.329	+0.328	+0.036	+0.388
+0.037	+0.477	+0.410	+0.210	+0.142	+0.076	+0.090	+0.230
-0.001	+0.240	+0.449	+0.193	+0.061	+0.274	+0.137	+0.163
+0.208	+0.197	+0.247	+0.326	+0.139	+0.109	+0.427	+0.312
		+0.096	+0.204	+0.299	+0.255	+0.228	+0.564

TABLE 3.—Root and stele measurements and breaking-tension data for 25 varieties and strains of winter wheat, 10 of which were repeated by space-planting the seeds by hand

[$\mu = 100$ except where noted]

Characteristic	Series ¹	Minhardt ²	Fulvio	Gladden ³	Trumbull	Thorne ⁴	Test No. 1029	Dawson ⁵	Test No. 1048	Test No. 1055
Diameter of root..... millimeters.....	5W	0.482±0.007	0.469±0.005	0.498±0.005	0.485±0.005	0.508±0.005	0.548±0.008	0.572±0.005	0.568±0.005	0.522±0.006
Diameter of stele..... do.....	6W	0.448±0.009	0.504±0.006	0.564±0.007	0.560±0.007	0.585±0.006	0.593±0.007	0.560±0.005	0.622±0.006	0.599±0.007
Cross section of root..... square millimeters.....	5W	0.371±0.006	0.348±0.005	0.390±0.004	0.355±0.004	0.384±0.005	0.420±0.005	0.433±0.005	0.439±0.004	0.397±0.006
Cross section of stele..... do.....	6W	0.290±0.007	0.379±0.005	0.434±0.006	0.410±0.007	0.456±0.006	0.464±0.006	0.419±0.006	0.468±0.006	0.449±0.005
Breaking tension..... grams.....	5W	0.189±0.005	0.177±0.004	0.200±0.004	0.180±0.004	0.207±0.005	0.240±0.005	0.256±0.004	0.254±0.004	0.218±0.005
Breaking tension..... do.....	6W	0.165±0.007	0.205±0.005	0.254±0.006	0.253±0.005	0.270±0.005	0.281±0.007	0.250±0.007	0.303±0.006	0.285±0.007
Breaking tension..... grams.....	5W	0.113±0.008	0.069±0.003	0.123±0.003	0.104±0.003	0.118±0.003	0.142±0.003	0.153±0.003	0.155±0.003	0.129±0.004
Breaking tension..... do.....	6W	0.072±0.004	0.118±0.003	0.163±0.004	0.137±0.004	0.168±0.004	0.173±0.005	0.142±0.004	0.177±0.005	0.165±0.005
Breaking tension..... grams.....	5W	243.5±5.80	287.5±5.65	397.0±7.70	331.0±7.05	363.0±5.50	352.5±8.45	378.5±9.50	389.0±9.85	405.5±7.50
Breaking tension..... do.....	6W	173.4±6.67	312.0±8.85	400.0±9.96	257.0±8.40	310.5±8.96	349.0±8.00	337.7±11.47	395.5±8.20	350.0±8.45
Breaking tension: Per millimeter of root diameter.....	5W	503.5±13.4	611.5±12.2	788.0±15.8	684.0±14.5	709.0±16.1	642.5±16.3	651.5±15.9	687.0±17.8	770.0±14.4
Breaking tension: Per millimeter of stele diameter.....	6W	381.5±15.4	612.0±17.8	706.5±18.3	474.5±14.9	530.7±16.0	583.0±13.5	600.7±19.0	638.0±14.1	582.0±14.3
Per square millimeter of root cross section.....	5W	660±18.0	836±17.2	1,022±20.4	949±21.8	943±21.8	846±23.0	805±21.8	892±23.8	790±18.1
Per square millimeter of stele cross section.....	6W	581±23.5	822±23.7	920±24.4	653±20.2	682±19.6	755±18.0	805±25.9	844±18.9	743±18.1
Thickness of cortex..... millimeters.....	5W	1.367±0.049	1.672±0.042	2.081±0.049	1.813±0.051	1.709±0.048	1.518±0.046	1.472±0.037	1.362±0.044	1.498±0.048
Correlations:	6W	1.102±0.03	1.579±0.03	1.638±0.02	1.105±0.04	1.191±0.03	1.252±0.03	1.380±0.04	1.360±0.04	1.274±0.03
Breaking tension and root diameter.....	5W	2.344±0.05	3.114±0.00	3.380±0.08	3.450±0.116	3.230±0.100	2.552±0.04	2.574±0.04	2.364±0.04	2.416±0.06
Breaking tension and stele diameter.....	6W	2.597±0.131	2.856±0.111	2.776±0.190	2.060±0.077	1.953±0.05	2.690±0.02	2.485±0.04	2.304±0.09	2.244±0.04
Breaking tension and root cross section.....	5W	0.059±0.001	0.065±0.002	0.089±0.002	0.069±0.002	0.067±0.001	0.068±0.001	0.072±0.001	0.066±0.001	0.066±0.001
Breaking tension and stele cross section.....	6W	0.078±0.002	0.067±0.002	0.067±0.002	0.079±0.002	0.067±0.001	0.067±0.002	0.073±0.002	0.077±0.002	0.078±0.002
Breaking tension and root diameter.....	5W	+0.090	+0.207	+0.141	+0.311	+0.307	+0.177	+0.327	+0.088	+0.337
Breaking tension and stele diameter.....	6W	+0.159	+0.064	+0.145	+0.065	+0.066	+0.302	+0.271	+0.088	+0.159
Breaking tension and root cross section.....	5W	+0.163	+0.192	+0.162	+0.254	+0.335	+0.106	+0.244	+0.097	+0.312
Breaking tension and stele cross section.....	6W	+0.196	+0.180	+0.220	+0.121	+0.312	+0.307	+0.306	+0.049	+0.261
Breaking tension and root diameter.....	5W	+0.029	+0.193	+0.097	+0.314	+0.315	+0.216	+0.319	+0.088	+0.305
Breaking tension and stele diameter.....	6W	+0.166	+0.045	+0.104	+0.052	+0.045	+0.316	+0.244	+0.000	+0.104
Breaking tension and root cross section.....	5W	+0.184	+0.170	+0.207	+0.302	+0.302	+0.116	+0.244	+0.045	+0.320
Breaking tension and stele cross section.....	6W	+0.206	+0.314	+0.171	+0.066	+0.066	+0.309	+0.346	+0.031	+0.246

Characteristic	Series ¹	Test No. 1128 ⁶	Purdue No. 1	Purkof	Red Rock	Nittany	Canawa	Poole	Fultaster
Diameter of root..... millimeters.....	5W	0.536±0.007	0.517±0.005	0.468±0.005	0.509±0.007	0.602±0.007	0.520±0.005	0.555±0.006	0.588±0.005
Diameter of stele..... do.....	6W	0.521±0.006	0.389±0.004	0.364±0.005	0.440±0.006	0.464±0.006	0.397±0.005	0.424±0.004	0.454±0.005
Cross section of root..... square millimeters.....	5W	0.377±0.005	0.215±0.004	0.178±0.004	0.257±0.006	0.286±0.007	0.218±0.004	0.240±0.005	0.274±0.005
Cross section of stele..... do.....	6W	0.231±0.006	0.121±0.003	0.108±0.003	0.156±0.004	0.173±0.004	0.128±0.003	0.148±0.003	0.164±0.004
Breaking tension..... grams.....	5W	0.129±0.004	0.115±0.003	0.109±0.003	0.156±0.004	0.173±0.004	0.128±0.003	0.148±0.003	0.164±0.004
Breaking tension: Per millimeter of root diameter.....	6W	380.5±8.62	322.0±6.75	261.5±5.85	436.5±8.65	451.0±10.30	324.5±8.15	314.5±8.95	386.0±7.20
Breaking tension: Per millimeter of stele diameter.....	5W	707.5±18.1	615.5±11.3	550.5±12.6	708.5±16.2	748.0±16.4	617.5±17.0	556.0±15.7	651.5±12.5
Per square millimeter of root cross section.....	6W	603.5±16.6	829±15.6	719±16.8	998±21.5	977±23.1	825±24.7	737±21.8	851±17.7
Per square millimeter of stele cross section.....	5W	835±23.2	1,521±32	1,503±40	1,702±46	1,620±42	1,532±51	1,208±38	1,430±33
Thickness of cortex..... millimeters.....	6W	1.485±0.048	2,750±66	2,596±80	2,970±90	2,738±83	2,740±117	2,218±78	2,432±74
Correlations:	5W	2.949±112	0.068±0.002	0.058±0.001	0.067±0.001	0.070±0.002	0.066±0.001	0.068±0.002	0.071±0.001
Breaking tension and root diameter.....	6W	0.073±0.002	+0.383	+0.057	+0.187	+0.288	+0.063	+0.291	+0.243
Breaking tension and stele diameter.....	5W	-0.058	+0.140	+0.152	+0.170	+0.177	-0.002	+0.169	+0.126
Breaking tension and root cross section.....	6W	-0.043	+0.345	+0.078	+0.145	+0.229	+0.072	+0.279	+0.168
Breaking tension and stele cross section.....	5W	-0.101	+0.333	+0.122	+0.179	+0.131	+0.056	+0.261	+0.116
Breaking tension and root diameter.....	6W	-0.134	+0.264	+0.122	+0.179	+0.131	+0.056	+0.261	+0.116
Breaking tension and stele cross section.....	5W	-0.096	+0.264	+0.122	+0.179	+0.131	+0.056	+0.261	+0.116
Breaking tension and root cross section.....	6W	+0.176	+0.264	+0.122	+0.179	+0.131	+0.056	+0.261	+0.116

$r > 0.195$ significant ($P < 0.05$), except where $n \pm 100$; $r > 0.254$ highly significant ($P < 0.01$), except where $n = 100$.

1 5W = sown with hand drill; 6W = seed space-planted by hand.

2 $n = 62$ in 6W.

3 $n = 92$ in 6W.

4 $n = 91$ in 6W.

5 $n = 73$ in 6W.

6 $n = 87$ in 6W.

TABLE 3.—*Root and stele measurements and breaking-tension data for 25 varieties and strains of winter wheat, 10 of which were repeated by space-planting the seeds by hand*—Continued

[p=100 except where noted]

Characteristic	Series ¹	American Banner	Test No. 1056	Test No. 1062	Hybrid se- lection 9	Hybrid se- lection 210	Hybrid se- lection 213	O. S. U. ² 122-12	O. S. U. ² 187-10
Diameter of root.....millimeters.....	5W	0.571±0.006	0.554±0.004	0.587±0.008	0.612±0.005	0.599±0.005	0.601±0.006	0.514±0.006	0.593±0.005
Diameter of stele.....do.....	6W	0.443±0.006	0.420±0.004	0.441±0.007	0.471±0.005	0.460±0.004	0.464±0.004	0.385±0.006	0.434±0.004
Cross section of root.....square millimeters.....	5W	0.259±0.006	0.245±0.004	0.274±0.008	0.290±0.004	0.279±0.004	0.284±0.005	0.211±0.005	0.252±0.005
Cross section of stele.....do.....	6W	0.160±0.004	0.142±0.002	0.158±0.005	0.178±0.003	0.168±0.003	0.173±0.003	0.121±0.004	0.153±0.003
Breaking tension.....grams.....	5W	372.0±11.0	321.0±6.35	340.5±10.00	351.5±8.80	373.5±8.85	401.5±8.25	341.0±8.30	347.0±7.70
Breaking tension: Per millimeter of root diameter.....do.....	5W	651.0±19.2	573.5±11.3	578.0±17.2	571.5±14.1	618.0±14.1	664.5±12.7	648.5±15.9	607.0±11.7
Per millimeter of stele diameter.....do.....	5W	838±24.6	763±17.4	774±23.2	737±18.2	802±18.6	856±16.4	886±22.6	792±16.2
Per square millimeter of root cross section.....do.....	5W	1,478±49	1,314±28	1,292±46	1,215±33	1,336±32	1,427±31	1,635±45	1,380±28
Per square millimeter of stele cross section.....do.....	5W	2,444±84	2,320±67	2,329±101	1,994±57	2,226±57	2,354±55	3,038±106	2,324±57
Thickness of cortex.....millimeters.....	5W	0.067±0.002	0.071±0.002	0.075±0.002	0.070±0.001	0.071±0.001	0.069±0.001	0.068±0.002	0.068±0.001
Correlations:									
Breaking tension and root diameter.....	5W	+0.155	+0.332	+0.134	+0.262	+0.341	+0.360	+0.317	+0.421
Breaking tension and stele diameter.....	5W	+0.237	+0.192	+0.252	+0.284	+0.272	+0.317	+0.248	+0.411
Breaking tension and root cross section.....	5W	+0.127	+0.348	+0.134	+0.296	+0.106	+0.370	+0.279	+0.459
Breaking tension and stele cross section.....	5W	+0.219	+0.174	+0.233	+0.317	+0.270	+0.319	+0.211	+0.399

¹ 5W = sown with hand drill; 6W = seed space-planted by hand.
² O. S. U. indicates Ohio State University (selection made at Columbus).

DISCUSSION OF DATA

FALL VERSUS SPRING SERIES

One of the main reasons for the 3W and 4W series was to obtain further data on the comparative values of fall and spring determinations on wheat varieties grown in the field. Mild weather with frequent showers during the fall of 1935 provided excellent growing conditions until near the end of November. In spite of somewhat late sowing (October 7), the plants entered the winter in good condition. The 3W series was run in December and the 4W series on samples from the same plots taken in March just as spring growth was starting.

In the 4W series it was not possible to measure the stele diameter with any degree of accuracy because of the confusion arising from the irregular collapse of the cortical tissue and the consequent interference with a clear demarcation of the stele outline. A comparison of the total diameter of the root in the 4W series with the stele diameter in the 3W series shows that the cortex was largely destroyed during the winter. Stele measurements on the 4W series were, therefore, discarded as unreliable.

The winter of 1935-36 was unusually severe, and both cold and heaving injury were apparent in the regular wheat nursery. The varieties included in this test, however, were not severely injured. Minhardi clearly showed the most damage from heaving. Comparison with data from earlier series is not easy, since no stele measurements had been made. However, the total root cross section in fall and in spring is available for four varieties in two seasons. The spring cross section in percentage of that found in the fall is given in table 4.

TABLE 4.—*Cross-sectional area of roots in spring in percentage of that in the fall for 4 winter wheats in 2 seasons*

Series	Season	Variety			
		Minhardi	Fulhio	Gladden	Purdue No. 1
W and 2W	1934-35	<i>Percent</i> 61.4	<i>Percent</i> 84.4	<i>Percent</i> 83.7	<i>Percent</i> 75.1
3W and 4W	1935-36	59.1	67.1	69.7	61.0

With the exception of Minhardi, the cortical tissue was retained to a much greater degree in 1934-35 than in 1935-36. Minhardi, because of its smaller and weaker growth, is more liable to damage, and probably nearly all the cortex was lost in both seasons. For Fulhio and Gladden the decrease in root cross-sectional area was roughly 15 percent from fall to spring in 1934-35, and over 30 percent in 1935-36. Purdue No. 1 showed approximately 10 percent greater decreases in both seasons.

Because the cortex is subject to this considerable and variable injury during the winter, measurements of root diameter are much better made in the fall. Furthermore, when the cortical tissue collapses it makes accurate measurement of the stele almost impossible by the method used.

One advantage to be gained by using samples taken in the spring would be a possibly greater range of values for the varieties due to differential injury in the winter. There are, unfortunately, disadvantages which more than offset the gain. It would be desirable to take plants from the field after danger of further injury is past, and yet before the growth has definitely started. Apparently, however, different varieties do not all start growth at the same time, and, furthermore, conditions causing injury may occur after growth has started. The time interval that elapses while a series is run becomes a serious factor, and even if this were reduced to a minimum by storing at temperatures near freezing, the varietal differences would still introduce considerable error. An example of how serious this may be is seen by a study of data for breaking tension in the 3W and 4W series given in table 1. In table 5, the differences between the fall and spring strength of roots are given.

TABLE 5.—*Difference in breaking tension of roots of winter wheat varieties in fall and in spring*

[Data from 3W and 4W series]

Variety	Breaking tension (grams) in—		Difference ¹	Variety	Breaking tension (grams) in—		Difference ¹
	Fall	Spring			Fall	Spring	
Minhardi.....	240.8	234.0	-6.8± 6.22	Thorne.....	394.5	422.5	+28.0± 9.49
Fulhio.....	396.2	417.8	+21.6±10.26	Baldrock.....	460.8	486.3	+25.5±10.33
Gladden.....	438.8	492.0	+53.2± 9.25	Purdue No. 1.....	323.5	344.3	+20.8± 7.44

¹ Spring breaking tension minus fall breaking tension.

With the exception of Minhardi, all these differences are significant, and, with the further exception of Fulhio, approach closely or exceed the 1 percent point. Apparently, Minhardi actually suffered injury and, in addition, was probably slow in resuming growth. The large increase in strength for Gladden seems most logically explained on the assumption that this variety resumed growth earlier or grew more rapidly than the others. The remaining four varieties were very similar in their behavior.

Probably during the course of the winter, assuming no periods when active growth is resumed, there is a decrease in root strength, varying with variety and with conditions. When growth commences again, this loss is made up, and with further development the roots continue to increase in tensile strength for some time. Varieties differ in the time and rate at which growth is resumed in the spring, and this is reflected in tensile strength of the roots. Data published in the previous paper support this assumption.

Even in the fall, determinations are subject to some error when samples are taken over a period of time. The 5W and 6W data (table 3) are a case in point. These rather extensive series were started December 11; the 5W was run first and was completed on January 9. The 6W followed and was completed January 18. By December 11 growth had probably stopped, but during mild weather late in the month and early in January, certain lines, notably the named varieties

originating in Ohio and the best adapted selections, made some further growth.

Other factors, however, enter the picture, and there are differences between these two series which cannot be ascribed to the time factor alone. Variability of soil may have been responsible for some of these differences, but the plots were within 100 feet and the soil was apparently uniform. The hand-spaced seed must have been sown deeper than the other, as it was a day or two later coming up. This was unfortunate, as wet weather had delayed planting, and it was October 5 when this seed went into the ground. Before the end of the month moderately severe frosts had occurred, and the growth was probably very slow afterward. Consequently, active growth stopped just about the time the secondary roots were becoming well established, and the disadvantage of the 6W series is reflected in lower tensile strength for some varieties.

Since in neither series was the seed planted very deeply, the secondary or permanent roots arose at a point very close to the seed, and, therefore, close to the primary roots. In the 6W series it is certain that some primary roots were selected. These do not show the varietal differences evident in secondary roots, as was clearly shown in the S and 2S series. Characteristics of the primary roots are different; the stele is smaller and the cortex thicker. Their inclusion has been a serious disturbing factor, and this, combined with the fact that the permanent roots had not reached a stage of development sufficiently advanced for best differentiation between varieties, greatly reduces the value of the results. The object in including the 6W series, as previously mentioned, was to see whether spade planting might not reduce variability. Because of the disturbing factors just mentioned, the data are useless for the purpose, but are included here as illustrating the difficulties that may arise in using field-grown material for tests.

When all the data are considered, it may be concluded that the best time to take samples from the field is late fall, as soon as possible after active growth has stopped.

VASCULAR STELE MEASUREMENTS

The earlier work had shown that the cortex was almost certainly not a factor in either extensibility or strength of the root. This being so, diameter or cross section of the vascular stele should be a better measure of root size than the corresponding data on the whole root. In the 3W, 5W, 6W, and 2L series, both measurements were made, and the data have been presented in tables 1, 2, and 3. The stele diameter was somewhat more difficult to measure, but with a fairly strong light the outline was reasonably clear, provided the collapse of cortical tissue had not been too serious.

The coefficient of variability shows the stele measurements to be slightly more variable than whole-root measurements. This is due, however, to the smaller means rather than to greater range. Thickness of cortex had no correlation with stele diameter, and the range of stele diameters was nearly as great as that of root diameters. These relationships all suggest the stele as the better measure of root size to associate with breaking strength.

The tensile strength of roots varies considerably. Besides size, others factors influence the breaking tension. The earlier studies⁵ showed:

When permanent roots start, they grow very rapidly and are characterized by large diameter except very near the growing tip. Changes that take place later are concerned with lignification of the vascular tissue and endodermis. The extent to which this thickening proceeds is determined by the length of time the plant continues to grow, and during this time little or no change in diameter takes place, except possibly a reduction due to collapse of cortical tissue.

Obviously the relationship between size and strength cannot be very close under these circumstances. Still, if there is a correlation, even of small magnitude, it would be of service in indicating the value of the different size measurements. To this end, breaking tension has been recorded directly, and in addition calculated in grams per millimeter of root and stele diameter, and in grams per square millimeter of root and stele cross section. Correlations between breaking tension and each of the four root measurements were also calculated. All these data are given in the tables.

The coefficient of variability offers a satisfactory means of comparing the different methods of calculating breaking tension. In table 6 these coefficients are given for the 3W series. This series was selected because it undoubtedly provides the most reliable data. No marked improvement was obtained when the simple breaking tension was corrected to any of the root measurements. Stele diameter gave the best results, and stele cross section the worst. This is disturbing because the cross section theoretically is the more logically associated with strength. However, a study of figure 1 throws some light on the situation.

TABLE 6.—*Coefficients of variability for certain of the data on the 3W series presented in table 1*

Characteristic	Min-hardi	Fulhio	Glad-den	Thorne	Bald-rock	Purdue No. 1	Average
Breaking tension.....gm.	27.8	22.4	22.3	26.0	24.2	25.8	24.8
Breaking tension*							
Per millimeter of root diameter....do....	25.9	25.8	21.8	22.3	20.6	23.6	23.3
Per millimeter of stele diameter....do....	24.7	24.8	22.6	21.4	20.3	23.2	22.8
Per square millimeter of root cross section.....do....	26.5	27.5	27.4	23.5	21.2	26.1	25.4
Per square millimeter of stele cross section.....do....	28.9	29.6	29.8	28.1	23.9	29.1	27.7

This graph is constructed of dots representing the actual recorded diameter, and the calculated cross-sectional area for all the classes into which the root measurements fell. Deviations of these dots from a smooth curve are due to errors introduced because of the size of the unit of measurement and more refined measurements were felt to be impracticable because of the extra time that would be required, and because error is undoubtedly introduced by the assumption that root and stele are both true cylinders. There is no object in refining measurements to the point where these errors become relatively large. From a study of the figure it is also to be noted that for the stele, using the cross section reduces the range of values considerably, and while the mean is also smaller, the reduction is not proportional.

⁵ See p. 31 of reference cited in footnote 2.

It is further evident from figure 1 that within the range of values encountered for stele diameter, and even for root diameter, the area plotted against the diameter gives a curve that does not deviate appreciably from a straight line, and, therefore, that using diameter introduces but little error, even though the true relationship may be linear with area rather than diameter.

Considering the correlations, much the same argument applies. Stele diameter gave the best values and was probably superior to stele cross section. At least there is every indication that it is fully as satisfactory. The r values were lower in the latter case because there was not a reduction in standard deviation proportional to the reduction in range.

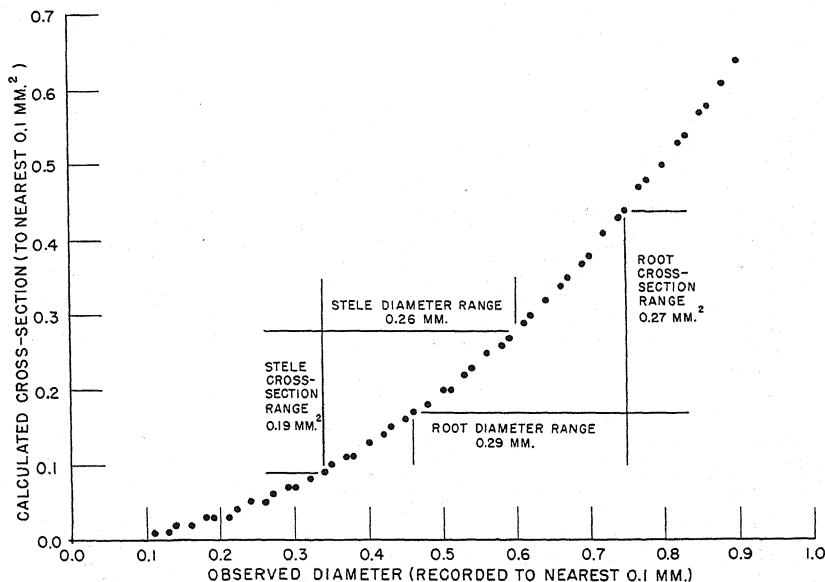


FIGURE 1.—Relation between diameter (measured to nearest 0.016 mm. and recorded to the nearest 0.01 mm.) and cross section of roots (calculated to the nearest 0.01 mm.²), and the range within which approximately 95 percent of the 3W series determinations (table 1) fell.

It has already been pointed out that growth conditions were not so good for the 5W and 6W series, and the results in general were more erratic. This was particularly true of the correlations presented in table 3. A study of scattergrams indicated that often one or a very few roots in the group of 100 would have large divergence from the mean, and would contribute a large proportion of the ($\Sigma dx \cdot dy$), or would reduce this total markedly. In several cases this gave abnormal r values, and the apparent inconsistencies can be discounted for this reason. The inadvertent inclusion of primary roots for some varieties was another disturbing factor.

As a whole, the data bear out the original premise that the stele is the best measure of root size to use. Stele diameter is more practical than stele cross section because of the difficulties involved in making sufficiently precise measurements to obtain accurate areas, and no serious errors are introduced by using it.

INFLUENCE OF FERTILITY LEVEL

If a test for resistance to heaving is to have any significance, it must rank varieties for a wide range of environment. No two seasons or fields are alike, and each presents its own specific set of conditions. The earlier series indicated that varieties fell in essentially the same order in several seasons. The 3W, 4W, 5W, and 6W series give further evidence that the varietal differences are consistent from 1 year to another. All these tests, however, were conducted on soil in a highly productive state. The levels of fertility rotation afforded an opportunity to study the effect of a range of soil productiveness on root characteristics. A very brief series (L series) was run on two varieties from this rotation in 1934-35, and indicated that there might be irregularities. Consequently, the 2L series was undertaken to see whether or not complications were to be anticipated from this source. Data are presented in table 2.

With but few exceptions, the results are consistent. As fertility level is raised, the roots become larger and stronger. Increased size is evident in stele as well as in whole root. However, Minhardi is distinctly out of line in breaking tension at the A (low) level. Development of the plants was very poor and secondary roots very young; in fact, in many cases they had not yet appeared. A considerable proportion of the roots used were seedling or primary roots, and this was obviously a serious disrupting factor. If this A-level figure is disregarded, the remaining data are reasonably consistent with those for the other varieties.

Even when well-adapted varieties of wheat are grown, farmers in Ohio still suffer losses from heaving. The severity of the loss depends in part on whether or not the soil lies wet in early spring, but the start that the plants have had in the fall is also very important. Since on these levels-of-fertility plots everything was sown on 1 day, the great difference that fertility level can make is clearly evident. The mean breaking tension for seven varieties (Minhardi omitted) was as follows:

	Grams
At the A level (no fertilizer)	271.5
At the B level (1 increment of fertilizer)	319.9
At the C level (2 increments of fertilizer)	361.7
At the D level (4 increments of fertilizer)	420.5

This is a wide range of values, and represents real differences in ability to withstand certain types of winter injury. It emphasizes the importance of giving wheat a good start in the fall. Yields on poorly fertilized fields are undoubtedly reduced more by winter injury than are those on more productive soils. Table 7 gives the mean yields of

TABLE 7.—Mean grain yields of varieties of winter wheat at four fertility levels in 6 seasons

Fertility level ¹	Average yield per acre for 11 varieties						Average for 8 varieties, 1935-36
	1928-29	1929-30	1930-31	1931-32	1932-33	Average	
	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>	<i>Bushels</i>
A	15.3	7.5	27.9	19.6	6.3	15.3	6.3
B	20.8	16.8	43.3	32.5	23.3	27.3	25.3
C	26.3	27.7	54.3	37.5	32.6	35.7	33.6
D	27.4	39.1	56.1	33.2	37.7	38.7	42.2

¹ See footnote, table 2.

the fertility-level plots for several seasons. It is obvious that the injury varies from 1 year to another, although in no case was any appreciable number of plants actually killed. It is not necessary, however, for plants to be killed in order that yields may be affected.

Apparently there is no reason to anticipate serious error in applying results from a test of root size and strength to a rather wide range of fertility conditions. Injury is greater at the lower levels, but the rank of varieties is not seriously changed. The series show that soil productiveness can be a very potent factor in fall development of roots. The benefits of fertilizer applications made at planting time include insurance against losses from winter injury.

SUMMARY

This paper presents data from five series of root studies, supplementing results already published. The principal points investigated were: (1) The time of year at which determinations can be made to best advantage on field-grown material, (2) the value of vascular stele measurements, and (3) the influence of fertility level on results.

Late fall was found to be the most satisfactory time to make studies. One advantage is that plants do not change so much over the period of time necessary to run the experiments. A second important point is that vascular stele measurements are much more simply and accurately made in late fall since the cortical tissue has not then collapsed seriously.

Stele diameter was found to be a better measure of root size with which to associate breaking tension than diameter or cross-sectional area of the whole root. Although cross section of stele is theoretically the best value to use, it did not give as good results as diameter. This was probably due to the fact that it is not practicable to take data with sufficient precision to give really accurate areas. No serious error is introduced by using diameter, however, because over the range in which the values of these determinations fall, the relationship between diameter and area is essentially linear.

A wide range of fertility level does not appreciably alter the rank of varieties, although it has a marked effect on both size and strength of roots.

OCCURRENCE AND LONGEVITY OF ASCOCHYTA PISI IN SEEDS OF HAIRY VETCH¹

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INTRODUCTION

Partial to complete defoliation of plants of certain *Vicia* spp. is caused by *Ascochyta pisi* Lib.³ Since the disease often is initiated by spores arising from seed-borne mycelium the fungus content of any seed stock materially affects its planting value. The association of *A. pisi* with seeds of hairy vetch, although not recognized by Orton (8),⁴ has been mentioned previously by Sprague (11). An *Ascochyta* has been described on pods of hairy vetch (*Vicia villosa* Roth.) or of other *Vicia* spp. (2, 12), but specific information regarding seed infection is almost entirely wanting. According to Allescher (1, p. 668) and Saccardo (10, p. 303) a fungus designated as *Ascochyta viciae* Lib. was recognized in Europe as a caulicolous pathogen of vetch but was not mentioned as being seed borne. Another species (*A. vicicola* Sacc.) was described from both leaves and pods (10, p. 303) of *Vicia sepium* L., while *A. pisi* was not listed as a pathogen of any species of *Vicia*.

Sprague (11) isolated *Ascochyta pisi* from 9-year-old seed of *Vicia faba* L.; but while he stated that the fungus is associated with seeds of *Vicia* spp., he apparently secured no isolants from this source. Rathschlag (9), in studying the degree of specialization of *A. pisi* from *V. faba*, was not concerned with the occurrence of the fungus in this or other species of *Vicia*.

Wolf (14) definitely proved that *Protocoronospora nigricans* Atk. and Edg. infects seeds of hairy vetch but failed to report the occurrence of *Ascochyta pisi*. Apparently he confined his studies to a few selected seed stocks since *A. pisi* is common in vetch seed produced in the Southern States. McKee and Schoth (?) in discussing the fungus diseases of vetch omitted any reference to seed infections.

Since the presence of *Ascochyta pisi* in seeds of vetches is a little-known or recognized phenomenon and the extent and nature have been mentioned only briefly (3), a few data from observations and experiments of the past 5 years are presented in this paper.

EXPERIMENTAL MATERIALS AND METHODS

No attempt was made in this study to secure seed stocks of hairy vetch from severely diseased plantings. With a few exceptions all of the samples were secured from commercial offerings or from imported

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² Prof. W. O. Gloyer suggested this problem and outlined the initial studies. Thanks are due to the official seed laboratories, importers, wholesalers, and retailers of forage seeds, and especially to Roland McKee, of the U. S. Department of Agriculture, for supplying the seed stocks.

³ The binomial, *Ascochyta pisi* Lib., rather than *Ascochyta* spp. has been used throughout the paper. While the specific identity of each isolation obtained in the present study was not positively determined, the size of the pycnospores indicated that either *A. pisi* as delimited by Jones (5), or the imperfect stage of *Mycosphaerella pinodes* (Berk. and Blox.) Stone, or both were the only species encountered. Many isolations were grown at various temperatures on natural and artificial substrates. Since a perfect stage was never observed it was assumed that only *A. pisi* was associated with seeds of *Vicia villosa*.

⁴ Italic numbers in parentheses refer to Literature Cited, p. 696.

seed stocks intended for commercial distribution. McKee drew subsamples from import lots originating in several European countries. The official seed laboratories of Arkansas, Maryland, New York, and Virginia supplied inspectors' collections and packets submitted by wholesalers. The origin of many seed-stocks could not be determined, and a number of samples represented blended bulks. In order that all sections of the United States would be represented, forage seed growers and wholesalers in several Southern and Pacific Coast States were requested to submit available stocks.

Representative pods were harvested from a few self-sown patches and commercial plantings of hairy vetch in the vicinity of Geneva, N. Y. A grower near Syracuse, N. Y., supplied tailings from his 1935 crop.

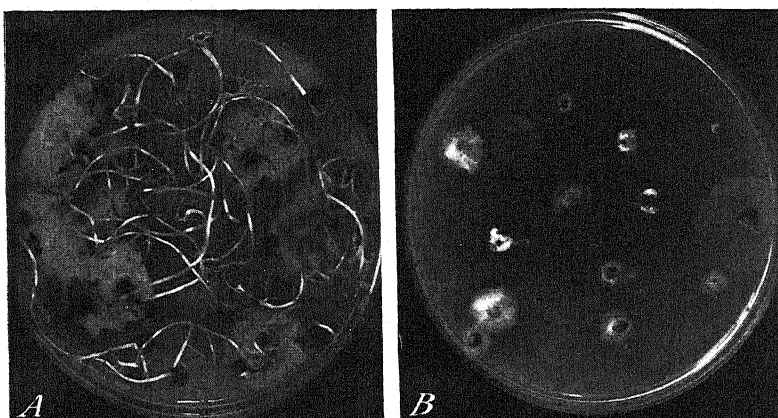


FIGURE 1.—*Ascochyta pisi* originating from surface-sterilized seeds of *Vicia villosa*: A, Normal seeds from a commercial seed stock; B, shriveled seeds from severely infected pods.

The samples were stored in a heated laboratory in their original containers. They were not fumigated or chemically treated in any manner for insect or fungus elimination. A few of the seed lots from Virginia, however, had been fumigated prior to shipment to Geneva. Portions taken from a few selected seed stocks were held at temperatures of -20° , 5° , 20° , and 35° C.

Ascochyta pisi vegetates sparsely and fruits tardily when vetch seeds are germinated on nonnutrient substrates used in seed laboratories. A mycelial growth or spore production comparable to that developing from infected peas has been observed only rarely.

Culture of surface-sterilized seeds in agar plates have made possible accurate quantitative determinations of *Ascochyta pisi*, since the fungus vegetates and fruits abundantly (figs. 1 and 2). In routine testing a mechanically counted lot of 50 seeds was surface-sterilized, placed on the agar surface with a flamed spatula, and spaced with a flamed transfer needle or by agitation.

Chloride of lime prepared according to Wilson's directions (13) or a commercial preparation of sodium hypochlorite was commonly used for surface sterilization. A chlorination period of 30 to 45 minutes with solutions containing 1.5 to 2.0 percent of available chlorine con-

trolled external fungi with a minimum of seed injury. A brief soak in a 0.1-percent solution of mercuric chloride eliminated surface-borne organisms but apparently inhibited growth of the internal fungi. Certain volatile mercury dusts were superior to chlorine as surface disinfectants, and their use expedited handling of the samples.

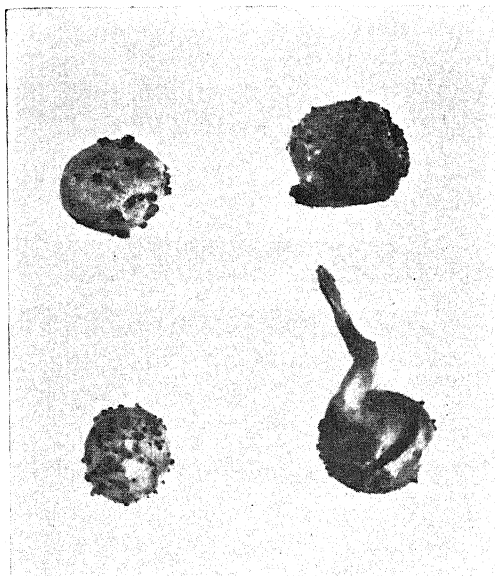


FIGURE 2.—Pycnidia and exuded pycnosporos of *Ascochyta pisi* on seeds of *Vicia villosa*. $\times 3$.

RESULTS

ASCOCHYTA PISI IN IMPORTED LOTS OF HAIRY VETCH SEEDS

In 1933 McKee submitted a group of 291 sub-samples of imported vetch seed for fungus determinations. The seed stocks were grown in, or exported from, several European countries during 1929, 1930, 1931, and 1932. At least 900 seeds from each sample were grown on agar plates during 1934. Only 1 of the 181 seed stocks imported from 10 countries during 1929 and 1930 was shown to contain *Ascochyta pisi*. As shown in table 1, 3 countries were

the principal exporters to the United States in 1931 and 1932. The presence of *A. pisi* was demonstrated in seed from only these countries, and from a fourth country from which seed was imported in 1932 only.

Seed of the 1933 crop from Hungary received at Geneva in March 1934 produced fungus colonies in October 1934 while seed from Lithuania did not.

TABLE 1.—*Ascochyta pisi* in seeds of *Vicia villosa* imported from several European countries

Origin of shipment	Entries of 1931			Entries of 1932		
	Samples received	Samples infected		Samples received	Samples infected	
		January 1934	October 1934		January 1934	October 1934
	Number	Number	Number	Number	Number	Number
Czechoslovakia.....	1	0	0	2	0	0
Germany.....	19	0	0	8	2	0
Hungary.....	32	2	0	4	0	0
Latvia.....	14	4	0	10	7	5
Lithuania.....	0	0	0	3	1	0
Sweden.....	3	0	0	2	0	0
All others.....	12	0	0	0	0	0

ASCOCHYTA PISI IN COMMERCIAL SEED STOCKS

The results obtained from growing surface-sterilized seeds received from official laboratories, wholesalers, and other agencies are summarized in table 2. The origin of many seed stocks was unknown. Several were mixtures of imported lots, and others were compounded from imported and domestic lots. This is especially applicable to the New York samples, as it was definitely known that many of them were imported, and in 1936 at least 18 were labeled as being representative of European-grown winter vetch. All but one of the New York samples produced growths of *Ascochyta pisi*, and the percentage of infected seeds varied from 1 to 4. Several lots of domestic origin contained 2 to 4 percent, and one lot at least 9 percent, of infected seeds.

A grower in central New York annually submitted a marketable sample for germination and disease determinations. *Ascochyta pisi* was isolated each year. The appearance of the seed did not suggest internal infections. Heavy rainfall in late spring apparently increased the percentage of fungus-seed associations, especially when the rye support crop failed to prevent matting of the vines.

TABLE 2.—*Presence and longevity of Ascochyta pisi in seeds of Vicia villosa stored in a heated laboratory since receipt at Geneva, N. Y.*

State where seed was grown or sold	Year when harvested	Samples received	Samples containing <i>A. pisi</i> in tests in—							
			March 1934	November 1934	September 1935	July 1936	April 1937	November 1937	April 1938	November 1938
		Number	Number	Number	Number	Number	Number	Number	Number	Number
Arkansas.....	1935	6	—	—	1	1	—	1	—	0
Illinois.....	1935	1	—	—	0	0	—	0	0	—
	1936	2	—	—	—	1	1	—	0	0
Maryland.....	1934	22	—	18	—	13	2	1	—	—
	1932	2	1	0	—	2	0	0	—	—
Michigan.....	1933	8	5	4	3	2	—	—	1	—
	1935	2	—	—	2	—	2	—	—	1
	1936	2	—	—	—	2	2	—	2	—
	1931	5	—	2	—	1	1	1	—	—
	1932	18	13	12	—	8	—	—	5	—
	1933	17	—	10	10	6	4	—	3	2
New York.....	1934	8	—	—	8	8	—	—	7	7
	1935	29	—	—	—	24	—	24	—	15
	1936	15	—	—	—	—	11	—	11	12
	1937	2	—	—	—	—	—	—	2	2
	1935	4	—	—	1	1	—	0	—	—
Oregon.....	1936	1	—	—	—	—	0	0	—	—
South Carolina.....	1933	2	2	2	1	2	—	0	—	0
Virginia ¹	1932	4	2	3	—	—	0	0	—	—
Do. ²	1932	11	8	6	—	3	—	2	—	—
Do. ³	1932	18	10	8	—	4	—	0	0	—
Do. ⁴	1933	3	1	0	—	0	—	—	—	—
Do. ⁵	1933	22	13	5	—	—	—	0	—	—
Do. ⁶	1933	4	4	1	—	1	1	0	0	—
Wisconsin.....	1933	3	—	3	—	1	0	0	—	—
Importers ⁷	1934	13	—	—	5	4	—	2	—	0

¹ Seed of European origin.

² Seed grown in Virginia or adjacent States, not fumigated.

³ Seed grown in Virginia or adjacent States, fumigated.

⁴ Samples obtained from importations from Hungary.

⁵ Samples obtained from importations from several European countries.

⁶ Seed received in Virginia in spring of 1934, origin unknown.

⁷ Samples obtained from 13 importers of European seed.

The data in table 2 show that several States either produce or plant infected seed. That *Ascochyta pisi* is particularly common to the seed in any State or States cannot be proved. In a few samples from

Maryland, believed to represent imported seed stocks, the percentage of fungus-seed associations exceeded 10. Infections in 15 percent of the seeds were noted in 1 imported and in 1 locally grown sample collected in Virginia. Since the optimum temperature for vegetation, spore production, and germination is above 25° C., it appears logical that the disease would be more severe in the Southern than in the Northern States. The fungus, however, develops at lower temperatures, and the percentages of infected vetch seedlings in fields in New York have always increased during winter or early spring. Undoubtedly moisture is more often a limiting factor in pod infections than is temperature.

LONGEVITY OF ASCOCHYTA PISI

The absence of demonstrable fungus growths from European vetch imported in 1929 and 1930, as mentioned previously, is not conclusive evidence that *Ascochyta pisi* was not present originally. Presumably phenological conditions could vary sufficiently from year to year to cause differences in the pod and, therefore, seed infections. The assumption that fungus-seed associations were established but that the fungus later died out is more tenable. The data in table 1 are inferential evidence of this assumption as is also the fact that when the 1932 entries from Latvia were examined in 1937 *A. pisi* could not be demonstrated in a single seed.

The samples received in the period from 1933 to 1938 were tested soon after their receipt and again on the dates noted in table 2. In general there was a decrease in the number of infected samples at each succeeding examination. Apparently the fungus retains its viability in a few seeds for a period of at least 7 years but usually dies out of seed stocks held in heated storage within 4 or 5 years.

That the temperature of storage affects the longevity of *Ascochyta pisi* was indicated in one experiment. Portions of a well-cleaned seed stock grown in New York in 1934 and of an imported lot presumably harvested in 1934 were stored in Sealright containers at -20°, 0°, 20°, and 35° C. during January 1935. Both seed stocks were air-dried to a moisture content of about 9 percent. The former contained 3 percent and the latter 4 percent of infected seeds in tests in December 1934. When surface-sterilized and grown on agar in March 1938 the percentages of fungus-seed associations at the various temperatures for the New York sample were: -20°, 1.2; 0°, 2.5; 20°, 1.5; and 35°, 0.5. For the imported lot at the same temperatures, they were: -20°, 2.5; 0°, 3.5; 20°, 0.2; and 35°, 0.5.

The percentage of viable seeds in the samples did not decrease materially at the three colder temperatures, whereas at 35° C. the germinations were originally 95.5 and 93.5 percent, and in 1938 46.0 and 30.0 percent for the New York and imported samples, respectively.

In experiments with New York samples a fumigant, carbon bisulfide, apparently did not reduce the percentage of seeds producing growths of *Ascochyta pisi*. Fumigation of small quantities of vetch collected in Virginia, however, may have eliminated the fungus from a few of them. As shown in table 2 about 56 percent of the fumigated and 73 percent of the untreated samples developed colonies of *A. pisi* when tested about 20 months after harvest. The original percentages

of infected samples could not be determined. Since the germination of every sample approximated 90 percent in March 1934, the fumigant could not have materially affected the viability of the seed.

COMPARISON OF LONGEVITY OF FUNGUS WITH LONGEVITY OF SEED

The decline in percentage of culturable fungus growths was not paralleled by a corresponding depression in germination of the seed. As is evident in table 2 *Ascochyta pisi* inside the seed may die within only 2 or 3 years and rarely remains alive longer than 6 or 7 years. Furthermore, the number of growths obtained from 200 test seeds of any sample usually decreased at each successive date of testing. For example, samples Nos. 3319, 4010, and 5908 from Virginia contained 15, 15, and 6 percent, respectively, of infected seeds in March 1934; 8, 6, and 4 percent in November 1934; 0, 2, and 1.5 percent in November 1936; and 0, 0, and 0 in July 1937. The germination percentages on artificial substrates in March 1934 were No. 3319, 94; No. 4010, 96; and No. 5908, 95. In July 1937 the percentages were 88, 91, and 92, respectively.

An imported sample germinated 94 percent in 1932 and 88 percent in 1937, whereas the fungus content decreased from 15 percent in 1932 to 0 in 1936.

Many other seed stocks retained their vitality for a number of years even though *Ascochyta pisi* died out entirely. On the other hand the germination of a few samples dropped rapidly while the percentage of infected seeds remained more or less constant for several years.

McKee and Schoth (7) stated that properly dried seed suffers little or no decrease in germination in 5 years and often no decrease in a longer period. In tests at Geneva 9 lots from Virginia contained from 86 to 90 percent of viable seed after 4 years in heated storage. Another group of 21 samples stored for 5 years germinated from 9 to 98 percent, with an average of 71 percent.

RELATION OF IMPERMEABILITY OF SEED COATS TO FUNGUS-SEED ASSOCIATIONS

That hard seeds materially affect the percentage of fungus growths developing on agar plates has not been demonstrated. The seeds that remained impermeable to water in 14-day tests of several lots were clipped with an electric needle and tested for *Ascochyta pisi*. The fungus developed in several plates but more often did not, and developed only rarely from a higher percentage of seeds than the results from unselected seeds indicated.

Studies with a large bulk drawn from a local crop harvested in 1934 showed that the incidence of *Ascochyta pisi* was greater originally in the promptly germinating than in the hard seeds. In this particular seed stock the hardness of the seed did not increase the longevity of any internal fungus.

Hard seeds to some extent decreased the accuracy and duplicability of the data in table 2. In counting the number of fungus colonies and computing the percentage of infections, the number of impermeable seed was not usually considered. Since *Ascochyta pisi* rarely emerges until swelling has occurred, it is possible that clipping the hard seeds would have increased the apparent fungus-seed associations. However, when a considerable number of seeds remained impermeable during the 20-day test period, the percentage of infections was based on swollen seeds alone.

RELATION OF POD SPOTS TO INFECTED SEEDS

Obviously, the seeds harvested from pods free of pod spot should also be disease-free. However, the development of *Ascochyta pisi* from normal seeds void of any external sign or symptom of disease suggests the possibility of systemic infection. In order to prove the relation of pod spots to fungus-seed associations, collections of pods from two garden patches were separated into diseased and clean groups and the seeds examined for the presence of *A. pisi*.

The pods from 1 patch were collected before maturity, and consequently the seeds were so abnormal as to appear diseased. In a total of 103 seeds from 28 pods only 1 maintained its spherical shape upon drying and only this 1 germinated. As expected, none of the pods free of external spots contained infected seeds. However, *Ascochyta pisi* originated from only 4 of the 73 seeds harvested from diseased pods. The 4 seeds were adjacent to spots covered with pycnidia. The use of a surface disinfectant apparently did not prevent the development of *A. pisi* since 2 infected seeds had been surface-sterilized, the other 2 had not.

Either hyphal masses or proliferated tissue from the inner walls of diseased portions of pods were removed and placed on agar surfaces. Scrapings from 20 pods seemed not to contain *Ascochyta pisi* although many colonies of *Penicillium* spp. and *Rhizopus nigricans* Ehr. developed.

In a larger collection from 14 plants it was found that 519 pods were spotted while 2,278 were not. After a period of 15 months the pods were again separated into groups based on the presence of definite lesions with numerous pycnidia, with very small lesions, and with no lesions. The seeds were carefully removed and classified as shown in table 3. They were mechanically counted in lots of 25, surface-sterilized for 25 minutes in a solution containing 1.8 percent of available chlorine, and spaced on surfaces of potato-dextrose agar.

The results (table 3) indicate that seeds are infected through the pods and that disease-free seed stocks can be obtained by pod selections. To assume that the presence of pod spots is proof that the seeds are infected is erroneous. Only 5.1 percent of the seeds of commercial size from pods with lesions were shown to contain *Ascochyta pisi*. Many of the pods were covered with spots and pycnidia, as many as 25 distinct spots being noted on several. If only 1 infected seed were obtained from a pod, the 5.1 percent shown in table 3 would have accounted for about 26.0 percent of the total number of spotted pods.

TABLE 3.—Germination and fungus content of vetch seeds obtained from pods spotted or not spotted with *Ascochyta pisi*

Condition of pods	Seeds cultured	Germination	Seeds giving rise to—	
			<i>A. pisi</i>	Contaminants
	Number	Percent	Percent	Percent
Small lesions, no pycnidia.....	225	50.6	0.88	1.33
Definite lesions, spherical seeds.....	500	76.0	5.10	1.80
Definite lesion, shriveled seeds.....	100	5.0	4.00	2.00
No lesions, hand-threshed seeds.....	500	71.0	0	1.20
No lesions, self-threshed seeds.....	200	81.5	0	.50

Presumably the seeds that escaped from self-threshed pods were more nearly mature than those threshed by hand since a higher percentage of the former produced normal sprouts. That fewer contaminants were obtained from this group might also be an indication of greater maturity.

LOCATION OF ASCOCHYTA PISI IN SEEDS

Wolf (14) demonstrated by a study of sectioned vetch seeds that hyphae of *Protocoronospora nigricans* may permeate all parts of the seed but did not mention the location, or even the presence, of *Ascochyta pisi*. When macerated portions of shriveled seeds were examined in the present study, hyphae of either *A. pisi* or *Alternaria* sp. or both were found in all parts of a few seeds. When *A. pisi* occurred in normally shaped seeds, it apparently was limited to the seed coats and the cotyledons. The plumules and radicles were not shown to be infected, and the fungus did not develop from them in agar platings. Furthermore, *A. pisi* rarely vegetates around ungerminated seeds and then only when they have swollen and the seed coats have become ruptured or deteriorated.

A number of seeds were soaked in water, and as soon as the seed coats had softened they were removed from the cotyledons. After a short period of sterilization in a chlorine solution both seed coats and cotyledons were placed on agar surfaces. Only 1 colony of *Ascochyta pisi* developed from the cotyledons of 250 shriveled seeds while 3 originated from the seed coats. Platings of entire seeds showed about 8 percent of infection.

Studies with other lots also proved that fewer fungus growths developed from surface-sterilized seed coats and cotyledons than from entire seeds. *Ascochyta pisi* vegetated around 1 decorticated seed and 1 seed coat in a subplot of 150 seeds of which 3 percent were previously shown to be infected. From another group of 50 seeds harvested from spotted pods 3 fungus colonies developed around the cotyledons while none originated from the seed coats. *A. pisi* vegetated around 5 percent of the unmutilated seeds. Surface sterilization apparently killed *A. pisi* in both the cotyledons and seed coats of several other test samples.

ELIMINATION OF ASCOCHYTA PISI BY HEAT TREATMENTS

Attempts to kill *Ascochyta pisi* either while inside the seeds or after it has emerged have not been highly successful. The results from heat treatments of dry or moist seeds were inconclusive. Immersion in water at 60° C. for 10 minutes reduced the percentage of seed infections without depressing germination, while a 13-minute soak killed the seeds but neither *A. pisi* nor *Alternaria* sp. was eliminated. Increasing the exposure period to 16 minutes effected complete kills of both seed and fungi.

When held in a drying oven at 70° C. for 9 days the germination of one lot of imported vetch was reduced from 92 to 85 percent, and after 16 days to 0. The presence of *Ascochyta pisi* was detected in seeds only to the seventh day. A domestic lot similarly treated was killed in 11 days, but pure cultures of *A. pisi* were secured from seeds treated for 12 days.

Steamed wheat grains harboring *Ascochyta pisi* were included in this experiment. In one group the percentage of grains producing growths of the fungus after 0, 1, 7, 11, 15, and 17 days were 80, 81, 37, 21, 29, and 0, respectively. The fungus was eliminated in 11 days from steamed grains with an originally higher moisture content. Pure cultures, however, were obtained from 23 percent of the grains exposed for 9 days.

Small samples from blended bulks collected locally were killed in 1 day at 90° C. No seed was demonstrated to contain living mycelium. Wheat grains, however, were sterilized only after an exposure of 5 days. Fragments of infected pods were also included in these trials. The pycnosporos which exuded promptly were placed to germinate at 25°. The percentages of germination for spores representative of the 0-, 2-, 5-, 7-, and 10-day periods were 98.5, 74.3, 37.5, 0.06, and 0.0, respectively.

Neither seeds, seed-borne mycelium, nor mycelium in dried wheat grains survived storage at 120° C. for 1 hour. An air temperature of 145° completely killed two samples of winter vetch in 30 minutes. Colonies of *Ascochyta pisi* did not arise from the surface-sterilized seeds. The viability of a well-dried domestic seed stock was depressed by exposure at 145°. The germination was 96 percent for the controls, 73 percent for the seeds exposed 30 minutes, and 0 for the seed heat-treated for 60 minutes. *A. pisi* also was destroyed in 60 minutes, but vigorous colonies originated from 2 percent of the seeds subjected to the 30-minute treatment.

CHEMICAL SEED TREATMENTS FOR CONTROL OF ASCOCHYTA PISI

The influence of certain chemical protectants on the germination of, and fungi in, vetch seed was studied with four infected lots. Similar portions of each seed-stock were treated with the materials listed in table 4 in December 1935. They were immediately planted in greenhouse beds either in soil previously cropped with wheat, in electrically heated soil, or in a mixture of washed sand and virgin muck, and also in the laboratory on wood-pulp blotting paper.

As shown in table 4, no chemical materially reduced the percentage of germinating seeds or emerged seedlings. Zinc oxide was definitely beneficial in regard to germination and to emergence and vigor of the seedlings.

Leaf infections were observed in a few rows, and might have developed in others had not the seedlings been discarded only 20 days after emergence. The appearance of *Ascochyta pisi* in the blotter tests also indicated that chemical dusts will not entirely prevent fructification of the fungus on the surface of seeds.

Shriveled seeds removed from a domestic bulk were treated with the chemicals listed in table 4. *Ascochyta pisi* developed on at least 5 percent of the seeds on every blotter irrespective of the kind or dosage of chemical. New Ceresan eliminated mold fungi and reduced, but did not eliminate, the percentage of *A. pisi*-bearing seeds. The other dusts were noticeably less efficient in mold control and effected no significant reduction in *A. pisi*-seed associations in this particular lot. The seeds were stored in sealed bottles; and when they were germinated again in October 1938, *A. pisi* was detected only on the seeds treated with 1.0-percent cuprous oxide and those treated with 1.5-percent zinc oxide.

TABLE 4.—*Relation of kind and dosage of fungicide to germination of seeds of hairy vetch placed on various substrates*

[Data are averages from four seed stocks]

Chemical and dose (percent)	Emergence from seeds planted in—			Germination on blotters	
	Common soil	Heated soil	Virgin muck	December 1935	October 1938
New Ceresan:	Percent	Percent	Percent	Percent	Percent
0.054 ¹	78	84	77	95	96
0.081.....	76	78	76	94	96
0.108.....	² 77	80	84	97	98
0.172.....	74	³ 78	80	95	95
Mercuric chloride:					
0.027.....	78	³ 82	79	94	96
0.054.....	84	85	76	98	98
0.108.....	82	85	² 74	⁴ 96	96
Copper carbonate: ⁵					
0.27.....	74	83	83	⁴ 95	93
0.68.....	77	84	81	97	⁴ 96
Cuprous oxide:					
1.00.....	77	80	77	⁴ 95	96
1.50.....	³ 73	87	77	⁴ 98	97
Zinc oxide:					
1.00.....	³ 82	87	88	98	97
1.50.....	² 85	87	87	97	96
Controls.....	76	83	78	96	⁴ 96

¹ Equivalent to a dosage of one-half ounce of dust per bushel of seed.² Seedlings infected with *Ascochyta pisi*.³ Several seedlings damped-off.⁴ *A. pisi* detected on seeds.⁵ Copper content stated to be 50 percent.

The influence of the various dusts on the development of *Ascochyta pisi* from four infected lots is shown in table 5. The seeds were treated in December 1935 and promptly stored in screw-top jars at room temperature. They were germinated on agar plates in June 1936. No material prevented the development of the fungus. New Ceresan, however, controlled the mold fungi, while the other chemicals were less efficient.

The data in table 5 further indicate that New Ceresan effected a significant decrease in the percentage of *Ascochyta pisi*-seed associations of the lots stored for 3 years. Presumably this reduction in fungus growths is the result of the action of gaseous ethyl mercuric phosphate during storage. The untreated seed was also stored in sealed bottles and was tested in October 1938. New Ceresan as a 0.2-percent dip did not affect the development of *A. pisi*.

At the beginning of this study chlorine soaks and ethyl mercury phosphate both as an instant dip and as a dust were compared with untreated seed to determine their effect on the presence of *Ascochyta pisi*. At least 200 seeds of each of 24 seed stocks were treated either by soaking for 90 minutes in 0.5-percent chlorine solution, by dipping in a 0.3-percent New Ceresan suspension in water, or by dusting with New Semesan Jr. *A. pisi* developed from 16 of the soaked, 10 of the dusted, 5 of the dipped, and 9 of the untreated tests. The average percentages of infected seeds were 2.6, 1.4, 0.7, and 1.0, respectively.

REDUCTION OF FOLIAGE INFECTIONS BY SEED TREATMENTS

Proof that leaf spot of vetch is initiated by seed-borne mycelium as well as evidence that it is difficult to control was obtained from a planting of 6 infected seed stocks in 1936 and 1937. Samples representative of both foreign and domestic seed stocks were selected for

their known fungus content and mechanically divided into 8 subsamples. They were treated either by soaking in hot water for the exposure periods listed in table 6, by dusting with Ceresan at the rate of 2½ ounces per bushel of seed, or by treating with a dust composed of 1 part New Ceresan and 4 parts cupric stearate by weight. The approximate adherence rate of this mixture was 4 ounces per bushel of seed. The treated lots were weighed to provide approximately 500 seeds for each row and were planted on August 8 in shallow trenches spaced sufficiently far apart to prevent dissemination of spores. Adjacent growths of hairy vetch originating from disease-free seed were not infected by *Ascochyta pisi*.

TABLE 5.—*Influence of kind and dosage of chemical on development of Ascochyta pisi and of saprophytic fungi in four seed stocks of hairy vetch treated in December 1935*

Chemical and dosage ¹ (percent)	Infected seeds, June 1936		Infected seed stocks, October 1938		Infected seeds, October 1938	
	<i>A. pisi</i>	Sapro- phytes	<i>A. pisi</i>	Sapro- phytes	<i>A. pisi</i>	Sapro- phytes
New Ceresan:						
0.054.....	3.0	1.0	1	1	0.5	0.5
0.081.....	3.5	1.0	1	1	1.0	.5
0.108.....	3.0	.5	0	1	.0	.3
0.162.....	3.0	.5	1	1	.4	.3
Mercuric chloride:						
0.027.....	4.0	9.5	3	4	4.0	10.8
0.054.....	7.0	10.0	4	4	3.7	7.5
0.108.....	6.0	8.5	3	4	1.5	6.8
Copper carbonate:						
0.270.....	2.5	6.7	3	4	4.8	6.7
0.680.....	6.0	2.2	3	4	3.5	10.7
Cuprous oxide:						
1.00.....	3.0	12.2	2	4	3.0	2.7
1.50.....	3.0	14.7	3	3	5.7	3.0
Zinc oxide:						
1.50.....	3.5	12.8	3	4	3.5	20.3
2.00.....	3.0	10.8	3	3	3.8	9.7
Stored untreated: ²						
Check.....	5.5	21.5	2	4	4.2	24.5
New Ceresan dip ³			4	1	5.8	.2
Sodium hypochlorite ⁴	5.0	1.8	4	2	4.8	.5

¹ Dosage is based on ratio of dust to seed expressed in percent by weight.

² Seed was stored untreated and then treated as indicated immediately before placing on agar plates.

³ A 0.2-percent solution containing 0.01 percent of the active ingredient, ethyl mercuric phosphate.

⁴ A solution testing 1.8 percent of available chlorine.

TABLE 6.—*Relation of hot-water soaks and of certain chemicals to the emergence, vigor, and leaf-spotting of plants of hairy vetch*

Seed treatment	Tempera- ture	Soaking period	Index of growth ¹	Length of stems	Plants infected	
					October	April
	°C.	Minutes		Inches	Percent	Percent
Untreated.....	50	50	100	12	1.8	9.5
	50	75	90	11	.1	2.1
	55	45	85	11	0	1.9
Hot water.....	55	45	70	10	.1	1.4
	55	60	65	9	0	1.7
	60	12	115	13	.6	8.3
Ceresan.....			100	10	0	5.7
New Ceresan ²			5	8	0	.8

¹ Comparative mass of vegetative growth in April 1937.

² Mixture of New Ceresan and cupric stearate.

Scattered leaf spots were noted in most of the rows of untreated seed during September, and in increasingly greater numbers each month until the plants were removed. All of the rows were examined critically during October and April. After the latter examination the plants were pulled and stored so that the progression of stem infections could be observed. The data summarized in table 6 are based on records of both field and dried-plant examinations for the six seed stocks. While they are only comparable, the data very clearly illustrate the influence of the various treatments upon the growth of, and disease present on, vetch seedlings.

OTHER FUNGI IN SEEDS OF HAIRY VETCH

While only *Ascochyta pisi* is commonly obtained from hairy vetch, other fungi are occasionally present and were encountered in this investigation. An unidentified species of *Alternaria* developed from surface-sterilized seeds in nearly 9 percent of the samples. In a portion of a domestic seed stock received in 1937, 52 colonies of this fungus originated from plantings of 400 seeds while only 12 colonies of *A. pisi* were recorded. Usually fewer seeds in any particular sample were infected with *Alternaria* sp. than with *A. pisi*. The former fungus was more tolerant of mercury compounds than the latter and often vegetated profusely even when *A. pisi* appeared to be injured.

Fusarium spp. were of infrequent occurrence in commercial seed stocks, being found only nine times. A planting at Syracuse, N. Y., produced heavily infected seed. Apparently moisture is very essential in the establishment of this seed-fungus association. It is, of course, probable that spores or mycelium were carried externally, as has been reported for peas (4, 5, 6), and were destroyed by surface sterilization.

Rhizoctonia solani Kühn was isolated from a single sample. The fungus was shown to be pathogenic to beans and peas as well as to several species of vetch. McKee and Schoth (?) reported that *Rhizoctonia* sp. seriously damaged plantings in Florida.

A mildly pathogenic fungus tentatively assigned to the genus *Xylaria* was secured from an imported seed-stock received in 1935. It completely destroyed seedlings growing on agar, while *Ascochyta pisi* under similar conditions merely discolored portions of the stems and infrequently invaded the tips.

FUNGI IN SEEDS OF OTHER VICIA SPECIES

Samples of a number of other vetches have been examined from time to time. *Ascochyta pisi* was not demonstrated in seeds of *Vicia atropurpurea* Desf., *V. dasycarpa* Ten., *V. ervilia* (L.) Willd., *V. monantha* Desf., *V. ludoviciana* Nutt., and *V. pannonica* Grantz. supplied by McKee. A single sample of *Vicia monantha* from South Carolina, and three samples of *V. atropurpurea* and two of *V. pannonica* from Oregon were proved to be infected.

Plants of *Vicia sativa* L. are susceptible to *Ascochyta pisi*, but the fungus was not isolated from any of 21 seed stocks. A typically clean sample dipped in a suspension of New Ceresan and then placed on an agar plate is shown in figure 3. *Alternaria* sp. was isolated from two samples and *Fusarium* sp. from one sample of *V. sativa* (fig. 4).

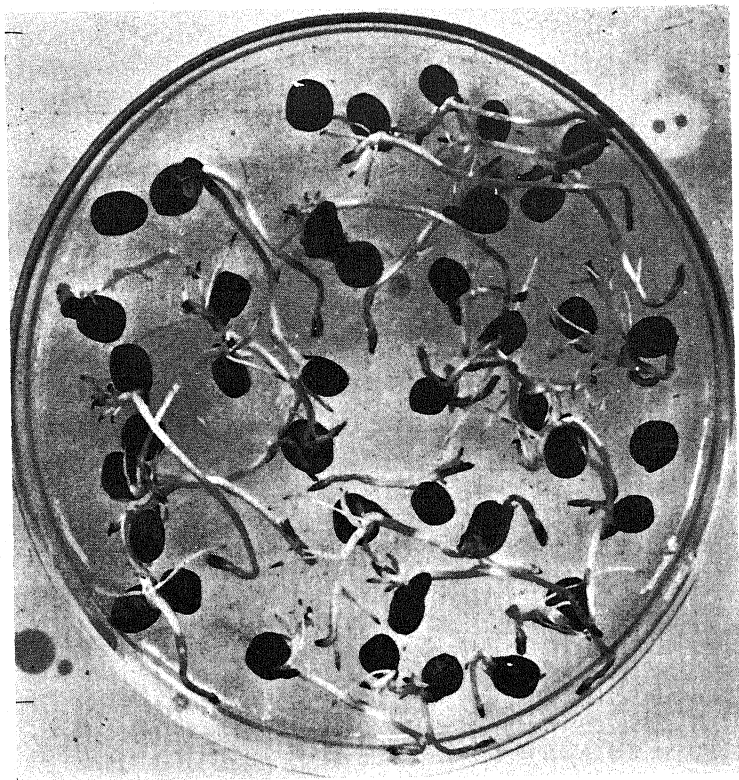


FIGURE 3.—Seeds of *Vicia sativa* surface-sterilized with New Ceresan. $\times 1$.



FIGURE 4.—*Alternaria* sp. originating from surface-sterilized seeds of *Vicia sativa*. $\times 2/3$.

SUMMARY

In 1934, 181 lots of hairy vetch seeds imported in 1929 and 1930, and 110 lots imported in 1931 and 1932 were surface-sterilized and cultured on agar plates. *Ascochyta pisi* was demonstrated in only 1 of the former; 16 of the latter seed-stocks showed infection in January 1934.

A total of 224 samples representative of both domestic and foreign crops of 1931 to 1937, inclusive, were secured from various agencies in 9 States. When tested immediately after their receipt, 149 of the samples were infected internally. During storage in a warm, dry laboratory for 1 to 5 years the number of *Ascochyta pisi*-seed associations decreased continuously.

The percentage of viable seeds remained nearly constant for 4 years or more, thus suggesting prolonged storage as a natural process of fungus elimination.

Longevity of the fungus could not be correlated with a hard-shell condition of the seed. A definite relationship existed between infection in pods and seeds.

The organism is usually resident immediately inside the seed coats but may occur in all parts of severely infected seeds.

Both spores and mycelium of *Ascochyta pisi* were less affected by exposures to hot air or water than were vetch seeds.

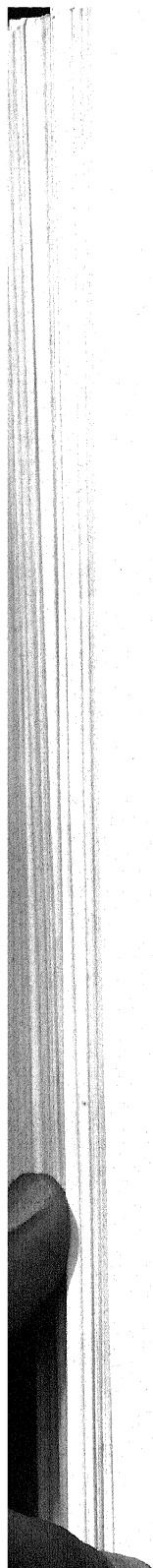
The fungus content of seed-stocks treated with New Ceresan decreased significantly during a 2-year storage period. Other chemicals were ineffective in eliminating internal fungi.

Alternaria sp., *Fusarium* spp., *Rhizoctonia solani* and *Xylaria* sp. were isolated infrequently from seeds of *Vicia villosa*. *Ascochyta pisi* may be resident in seeds of several *Vicia* species but not in those of *V. sativa*.

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FURTHER STUDIES OF THE EFFECTS OF TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS UPON THE PHOTOPERIODIC RESPONSES OF PLANTS¹

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INTRODUCTION

The marked effect exerted by temperature on the photoperiodic response of many of the higher plants has already been reported.² During the winter of 1937-38 an intermediate or "medium" temperature house with a minimum night temperature of 63° F. was maintained in addition to the "warm" house kept at 70° and the "cool" house kept at 55° which had been used the previous year. Most of the observations were made between October and April, when the day temperatures and the night temperatures remained relatively uniform. From the results obtained during the past several seasons it appears, as Hamner and Bonner³ reported for *Xanthium*, that night temperatures are more effective than the accompanying day temperatures in determining the nature of the response that many kinds of plants will make. So far, a control of only the night temperatures during the winter months has proved to be consistently effective in modifying the influence of photoperiod on plants.

EXPERIMENTAL PROCEDURE

In 1938-39 the minimum temperatures employed were 55°, 65°, and 75° F. This rise in the warmer-temperature houses was used to accentuate the effect of warm temperature in restricting the flowering of such short-day plants as tobacco and poinsettia.

Long- and short-day conditions were provided in each of the three temperature houses. The long-day environment was secured by the use of 100-watt lamps from just before sunset until midnight. These were so placed as to deliver from 30 to 80 foot-candles of light to the plants. Prior to November 1 and after March 1, short days of 9½ to 10 hours were maintained. No artificial shortening of the day was employed during midwinter.

Six or more plants of a variety were used in each treatment. The different lots received similar cultural and moisture treatments. When variability in growth within a lot was apparent, the plants that were photographed represent the average condition. In the lots that were relatively uniform representative samples were selected at random. As will later be shown, the amount of variability is largely a matter of the growth response of each species to a particular environment.

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² ROBERTS, R. H., and STRUCKMEYER, B. ESTHER. THE EFFECTS OF TEMPERATURE AND OTHER ENVIRONMENTAL FACTORS UPON THE PHOTOPERIODIC RESPONSES OF SOME OF THE HIGHER PLANTS. *Jour. Agr. Res.* 56: 633-677, illus. 1938.

³ HAMNER, KARL C., and BONNER, JAMES. PHOTOPERIODISM IN RELATION TO HORMONES AS FACTORS IN FLORAL INITIATION AND DEVELOPMENT. *Bot. Gaz.* 100: 388-431, illus. 1938.

Except as indicated, the data recorded for the plants in table 1 were obtained from 2 to 10 repeated plantings. The results from repeating a treatment have been very consistent. The reason for this is that care was taken to have plants from a similar source. It is to be shown later, for example, that plants from cuttings and from seedlings give unlike responses to photoperiod at certain temperatures.

It is believed that the length of time required for a plant to react to a photoperiodic treatment should be given relatively little weight in attempting to determine the response of a plant to photoperiod. The

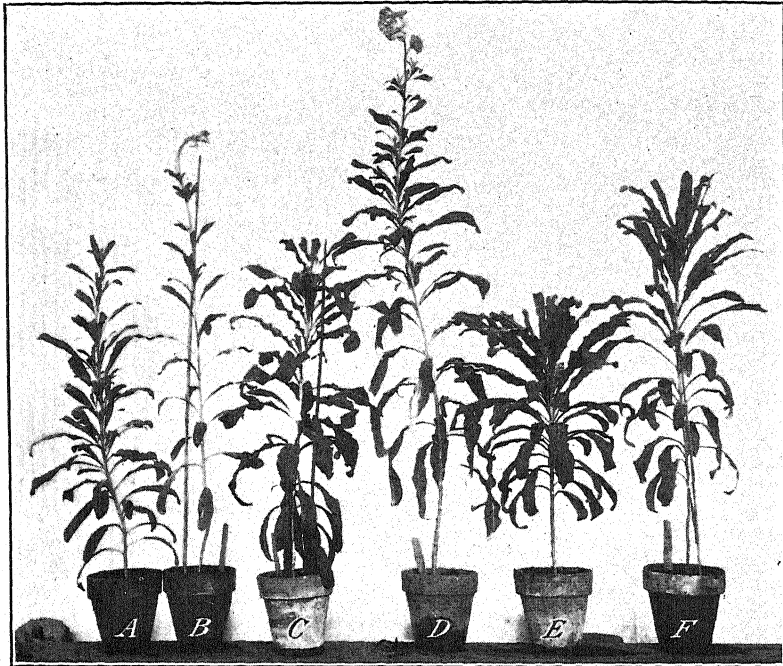


FIGURE 1.—Stock, which is a long-day plant at an intermediate temperature (D): A, Cool, short-day (budding); B, cool, long-day (blossoming); C intermediate temperature, short-day (nonblossoming); D, intermediate temperature, long-day (blossoming); E, warm, short-day (nonblossoming); F, warm, long-day (nonblossoming).

time required for a plant to flower, though sometimes very important commercially, is of little interest in cataloging the photoperiodic reactions of plants. The important item technically is not whether a plant comes to flower quickly but, rather, whether it soon acquires the growth characteristics that are readily recognized as being associated with blossom production. One lot of plants may come to flower much more quickly in one location than another lot in a different location, although blossom primordia may have been initiated at practically the same time in each lot. It seems confusing to speak of the first group as being more responsive to photoperiod than the second group. When classifying plants as to their photoperiodic type, the later as well as any immediately apparent reaction should have its proper emphasis.

EFFECT OF TEMPERATURE ON THE RESPONSE OF PLANTS TO PHOTOPERIOD

The addition of a third temperature environment produced a number of results which were not apparent in the earlier experiments where only two temperature levels were maintained. One example is that of stock, variety Xmas pink (fig. 1). This plant was a long-day plant

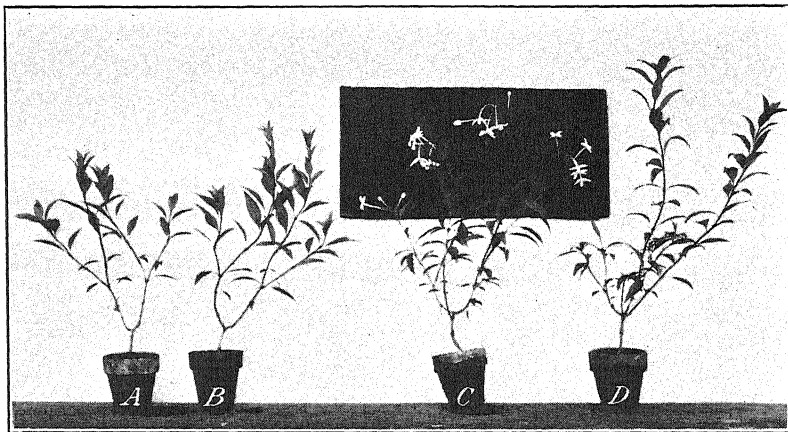


FIGURE 2.—*Bouvardia humboldti*, which blossoms quickest in warm, short days; plants in long days blossom at some growing points in longer periods of time; A, intermediate temperature, short day; B, intermediate temperature, long day; C, warm, short day; D, warm, long day.

when grown at an intermediate temperature. In a cool location the plants in short days as well as in long days blossomed, although those in short days were slower in coming to flower. Neither the lot in short days nor that in long days flowered in the warm location. Post ⁴

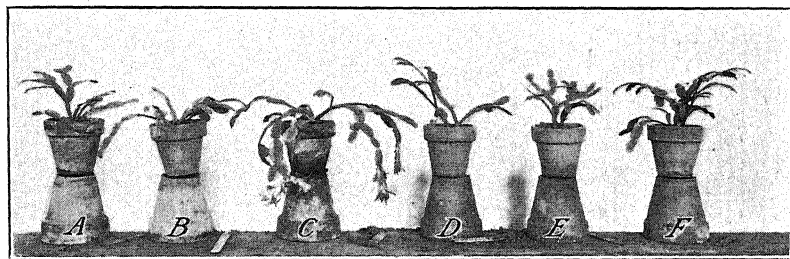


FIGURE 3.—Christmas cactus, which needs an intermediate temperature as well as a short day to induce early blossoming (C): A, Cool, short day; B, cool, long day; C, intermediate temperature, short day; D, intermediate temperature, long day; E, warm, short day; F, warm, long day. Plants in cool, long days sometimes form buds.

has previously called attention to the effect of temperature upon the reaction of stock to photoperiod.

The response of nasturtium, variety Golden Gleam, was similar to that of stock. Morning-glory, variety Heavenly Blue, blossomed in

⁴POST, KENNETH. SOME EFFECTS OF TEMPERATURE AND LIGHT UPON THE FLOWER BUD FORMATION AND LEAF CHARACTER OF STOCKS (*MATHIOLA INCANA*). Amer. Soc. Hort. Sci. Proc. 32: (v. 33): 649-652, 1935.

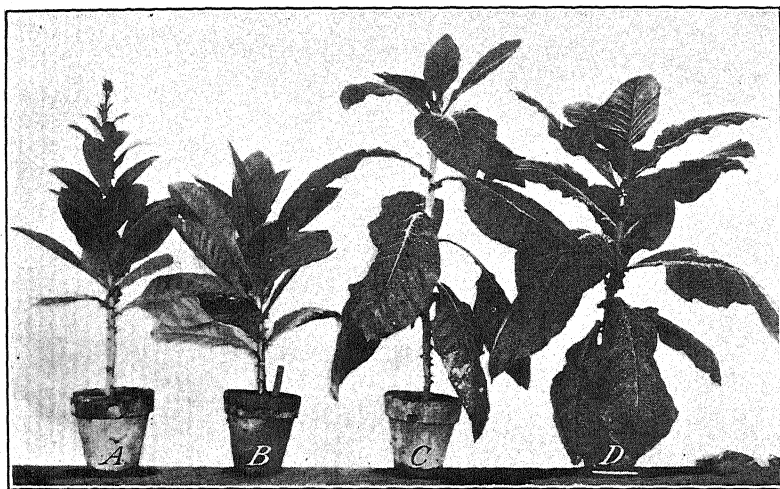


FIGURE 4.—Maryland Mammoth tobacco, which fails to fruit in short days if the temperature is too warm: A, Outdoors, short day (budding); B, outdoors, long day; C, greenhouse, short day (remaining vegetative); D, greenhouse, long day. Grown from seed produced by plants in long days at cool temperature. Photographed August 19.

short days at a warm-temperature, in both long and short days at an intermediate temperature, and in long days (after a long time) at a cool temperature. Similar effects of temperature in modifying the response to photoperiod were noted for a number of species, as table 1 and figures 2 and 3 show.

Maryland Mammoth tobacco is generally regarded as a strictly short-day plant. Apparently this view is the result of the plants hav-

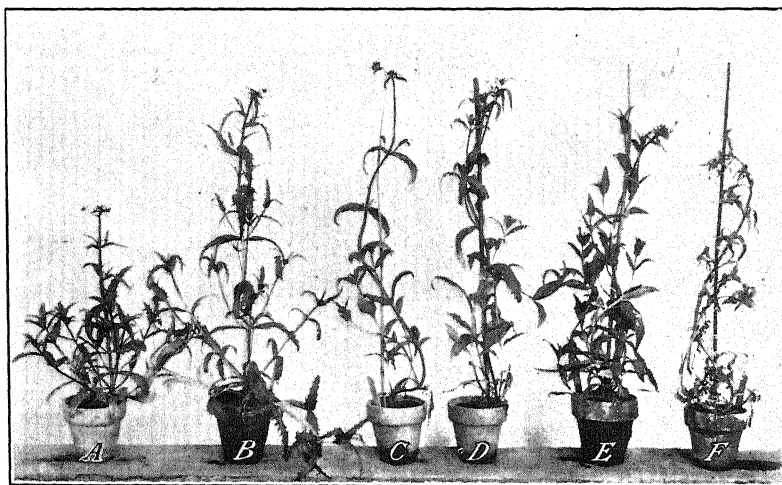


FIGURE 5.—*Nemesia versicolor*, which blossoms in a wide range of environments: A, Cool, short day; B, cool, long day; C, intermediate temperature, short day; D, intermediate temperature, long day; E, warm, short day; F, warm, long day. The plants at a warm temperature, especially in long days, show a chlorotic condition (F).

ing been grown under a limited range of temperature conditions, as this variety becomes fruitful in long days as well as short when grown at a minimum temperature of 55° F. Plants given short days at a very warm temperature remain vegetative and fail to blossom (fig. 4). Thus this variety not only fruits in long cool days, but fails to fruit in short days at a high temperature. Seed produced in cool long days grew into plants that gave typical short-day reactions at an intermediate temperature.

Nemesia versicolor (fig. 5) and annual larkspur (*Delphinium ajacis*) responded only slightly to photoperiod. The date of flowering was delayed at the cool temperature, but the time at which the type of

TABLE 1.—*Influence of temperature upon the photoperiodicity of some of the higher plants*

Plant	Nonfruitful (Nf) or flowers (F) produced at temperature of—						Remarks
	70° or 75° F. (warm)		63° or 65° F. (intermediate)		55° F. (cool)		
	Short day	Long day	Short day	Long day	Short day	Long day	
Alfalfa (<i>Medicago sativa</i>)	Nf	Nf ¹	Nf	F	F ²	F ³	3 clones.
Barley (<i>Secale cereale</i>)	Nf	Nf ¹	Nf	F	F ²	F ³	2 varieties.
Beet (<i>Beta vulgaris</i>)	Nf	Nf ¹	Nf	Nf ⁴	F ²⁴	F ³	3 varieties.
<i>Begonia semperflorens</i>	Nf	F	F	F	F ²	F ³	
Bluegrass (<i>Poa pratensis</i>)	Nf ¹	Nf	Nf	Nf	F ³	F ²⁴	
<i>Bougardia humboldti</i>	F ³	F ²	Nf	F	Nf ¹	Nf	See fig. 2.
Chinese cabbage (<i>Brassica pekinensis</i>)	Nf	Nf ¹	Nf	F ⁴	F ²	F ³	
Christmas cactus (<i>Zygocactus truncatus</i>)	Nf ¹	Nf	F ³	Nf	F ⁴	F ²⁴	See fig. 3.
<i>Chrysanthemum morifolium</i> var. Lillian Doty.	F ³	Nf	Nf	Nf	Nf ¹	Nf	
<i>Chrysanthemum morifolium</i> var. Golden Sceptre.	F ³	Nf	F	Nf	Nf ¹	Nf	
Cocklebur (<i>Xanthium echinatum</i>)	F ³	Nf	F	Nf	Nf ¹	Nf	10 weeks.
Corn (<i>Zea mays</i>)	F ²	F ³	F ^{4,5}	F	(⁶)	(⁶)	
Cornflower (<i>Centaurea cyanus</i>)	Nf	F	Nf	F	F ²⁴	F ³	
<i>Cosmos sulphureus</i> var. Klondike.	Nf ¹	Nf	F ³	Nf	F ⁴	Nf	
<i>Dianthus barbatus</i>	Nf	Nf ¹	F ²	F ³	F ⁴	F	
Dogfennel (<i>Anthemis cotula</i>)	Nf	F ³	Nf ⁴	F	F ²	F	
Gourd (<i>Luffa cylindrica</i>)	Nf	F ³	F ²	F	(⁶)	F ⁴	
Jimsonweed (<i>Datura stramonium</i>)	F ³	F ²			Nf ¹	Nf	
Kohlrabi (<i>Brassica oleracea caulorapa</i>)	Nf	Nf ¹	Nf	F ⁴	F ²	F ³	
Mallow (<i>Malva verticillata</i>)	F ³	Nf	F	F ²	Nf ¹	Nf	
Marigold (<i>Tagetes erecta</i>)	(⁶)	F	(⁶)	F ³	F ²	F	
Morning-glory (<i>Ipomoea purpurea</i>) var. Heavenly Blue.	F ³	Nf	F	F ²	Nf ¹	F ²	
<i>Naegelia cinnabarina</i>	F ³	F ²	F ⁴	Nf	(⁶)	(⁶)	1 lot.
Nasturtium (<i>Tropaeolum majus</i>)	Nf	Nf ¹	Nf	F ³	F ²	F	
Peas (<i>Pisum sativum</i>) var. Canada field.	Nf	Nf ¹	Nf	F ³	F ⁴	F	
Peas (<i>Pisum sativum</i>) var. Wisconsin Early.	(⁶)	Nf	Nf	F ⁴	F ²	F ³	
Pennycress (<i>Thlaspi arvense</i>)	Nf	Nf ¹	Nf	Nf	F ²	F ³	
<i>Petunia hybrida</i>	Nf	F	Nf	F ³	F ²	F	
Poinsettia (<i>Euphorbia pulcherrima</i>)	Nf ¹	Nf	F ³	Nf	Nf ¹	F ²⁴	
Primrose (<i>Oenothera</i> sp.)	Nf	Nf ¹	Nf	F ⁴	Nf	F ³	
Red clover (<i>Trifolium pratense</i>)	Nf	Nf ¹	Nf	F ⁴	Nf	F ³	
<i>Rudbeckia laciniata</i>	Nf	F ³	Nf	F	F ²	F	
<i>Salvia splendens</i> var. Harbinger.	F ³	Nf	F	Nf	F	F ²	
Snapdragon (<i>Antirrhinum majus</i>)	Nf	F	Nf	F ³	F ²	F	
Soybeans (<i>Soja max</i>) var. Biloxi.	F ³	Nf	F	Nf	Nf ¹	Nf	
Soybean (<i>Soja max</i>) var. "Chippewa"	F ²	F ³	F ⁴	F	Nf	Nf ¹	
Spinach (<i>Spinacia oleracea</i>)	Nf	Nf ¹	Nf	F	Nf	F ³	
Stock (<i>Matthiola bicornis</i>)	Nf	Nf ¹	Nf	F	F ²	F ³	See fig. 1.
Timothy (<i>Phleum pratense</i>)	Nf	Nf ¹	Nf	F ⁴	Nf	F ³	
Tobacco (<i>Nicotiana tabacum</i>) var. Maryland Mammoth.	Nf ¹	Nf	F ³	Nf	F	F ²	See fig. 4.
Vetch (<i>Vicia sativa</i>)	(⁶)	(⁶)	Nf	F	F ²⁴	F ³	
White clover (<i>Trifolium repens</i>)	Nf	Nf ¹	Nf	F ⁴	Nf	F ³	3 varieties.
White clover (<i>Trifolium repens</i>) var. Louisiana White.	Nf	F	F ⁴	F ³	F ²	F	Cuttings.

¹ Flowering suppressed by temperature.

² Flowering induced by temperature.

³ Typical photoperiod reaction.

⁴ Some tendency to flower.

⁵ Androgynous tassels are typical.

⁶ Plants fail to grow.

growth appeared that is characteristic of blossoming plants was not greatly different in the several environments. Plants of *Nemesia* acquired an etiolated appearance at the warm temperature, especially in long days.

It has commonly been observed that corn does not make normal growth in the greenhouse at temperatures of 60° to 65° F.⁵ At these

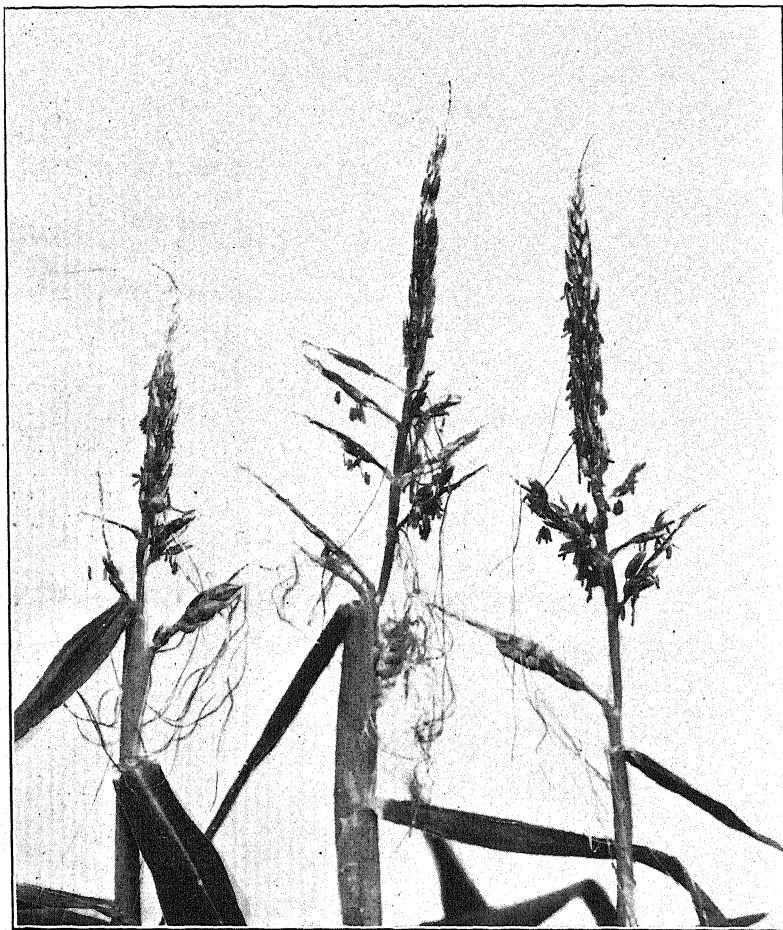


FIGURE 6.—Corn (Iowa Agricultural Experiment Station Selection No. 1445), which produced androgynous tassels at an intermediate temperature. Normal-appearing tassels and ears are produced at a warmer temperature.

temperatures plants in long days frequently produce androgynous tassels (fig. 6) and poor ears, and plants in short days develop abortive tassels but have more nearly normal-appearing ears. The variety Golden Glow as well as strains of sweet corn were very little affected by photoperiod at a warm temperature as normal tassels and ears were produced.

⁵ ROBERTS, R. H., KRAUS, JAMES E., and LIVINGSTON, NORMAN. CARBON DIOXIDE EXCHANGE RHYTHM AND FRUITFULNESS IN PLANTS OF DIFFERENT REPRODUCTIVE HABITS. Jour. Agr. Res. 54 : 319-343, illus. 1937

EFFECT OF OTHER FACTORS ON THE RESPONSE OF PLANTS TO PHOTOPERIOD

There are a number of factors besides temperature which modify the effect of photoperiod on the growth of plants, as, for example, moisture and mineral nutrition. These, however, have not been varied in the present studies. Two other items have been given some attention, namely, the source of the plants and the effect of starting the light treatments at different periods in the life of the plants.

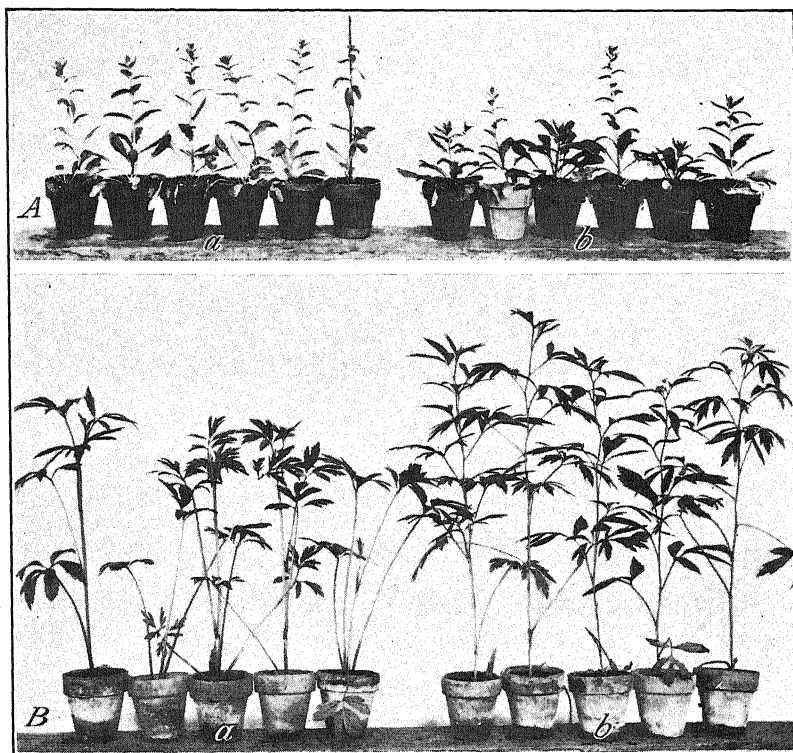


FIGURE 7.—Seedlings (A) or clones (B) may have a uniform or a variable population depending on the environment: A, Pennycress seedlings in cool, long-day (a) and cool, short-day (b) environments; B, *Rudbeckia laciniata* clones in cool, long-day (a) and warm, long-day (b) environments.

In another paper⁶ it has been reported that plants grown from cuttings taken from a flowering plant made a different response to the same photoperiod treatments than seedlings of the same species. It was found also that cuttings from nonflowering plants reacted more nearly like seedlings than did cuttings from flowering plants. The time of reaction to photoperiod was also altered by the use of root-forming hormones. Cuttings of *Antirrhinum*, *Centaurea*, and *Petunia* were grown with and without a root-inducing "hormone." The use of commercial Auxilin according to the printed directions increased the vegetative growth but somewhat delayed the flowering of these clones.

⁶ STRUCKMEYER, B. ESTHER, and ROBERTS, R. H. EFFECT OF PHOTOPERIOD AND TEMPERATURE UPON THE GROWTH OF SEEDLINGS AND CUTTINGS. In press. Amer. Jour. Bot.

Both clons and seedlings may be either variable or uniform depending on the environment in which they are grown (fig. 7). This variability can neither be predicted nor avoided in some locations. The production of a uniform population is obviously a matter of having a variety that is adapted to the treatment to be used.

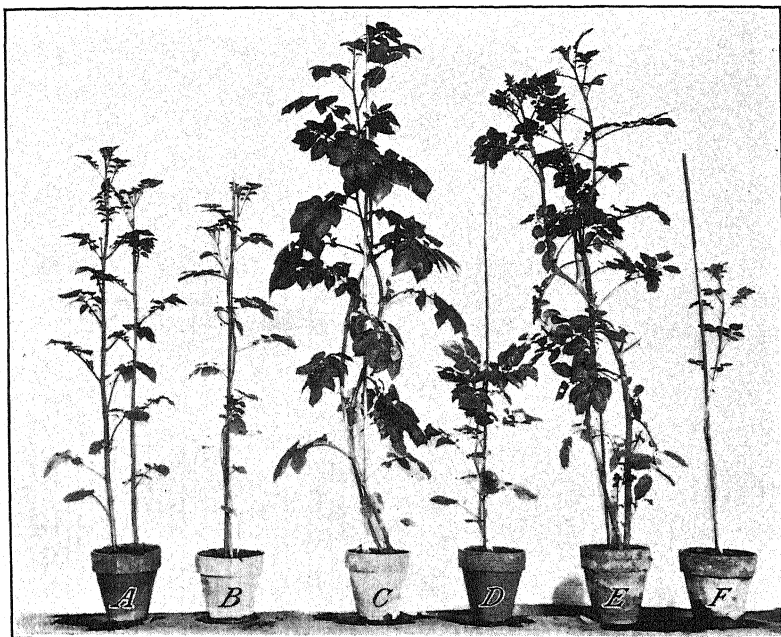


FIGURE 8.—The influence of the size of potato seed pieces on the growth of the plant is modified by the environment: A, Large piece, medium temperature, long-day; B, small piece, medium temperature, long-day; C, large piece, medium temperature, short-day; D, small piece, medium temperature, short-day; E, large piece, warm, long-day; F, small piece, warm, long-day. Like warm-temperature plants, the medium-temperature plants responded to photoperiod in short days, but like cool-temperature plants, made little response in long days.

Another instance of the effect of environment on photoperiodic influences was found in connection with the source of seed. When large seed-potato pieces (average 96.0 gm.) and small ones (average 7.0 gm.) were planted in warm long-day location, the plants from the large pieces were much more vigorous than those from the small pieces (fig. 8). This result was not consistent in all environments, however, as there was little effect of size of seed piece in a cool location. Similar results were secured in four tests.

The size of the corn kernel planted markedly effects the vigor of the seedling in a cool location, as the large kernels produce much larger plants than the small ones (fig. 9). In a warm location there is little influence from size of seed.

Another factor affecting the response which a plant makes to a photoperiod environment is, as Knott⁷ has pointed out in the case of spinach, the stage of growth the plants are in at the time the treatment is begun. To secure the most desirable type of growth, many

⁷ KNOTT, J. E. THE EFFECT OF TEMPERATURE ON THE PHOTOPERIODIC RESPONSE OF SPINACH. N. Y. (Cornell) Agr. Expt Sta. Mem. 218, 38 pp., illus. 1939.

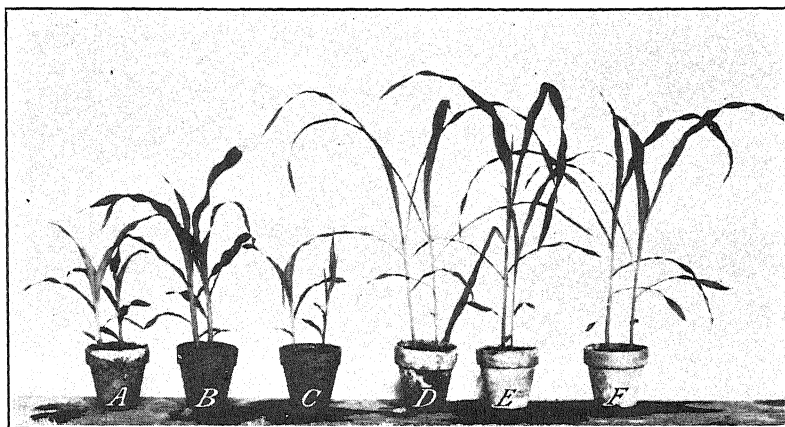


FIGURE 9.—Large corn kernels produce stronger plants (*B*) than small kernels (*C*) when environment is cool, but no appreciable response is obtained when it is warm (*E*, *F*): Plants from medium-sized (*A*), large (*B*), and small (*C*) kernels planted out of doors in May; plants from medium-sized (*D*), large (*E*), and small (*F*) kernels planted in the warm greenhouse.

plants appear to require a change in environment as growth progresses. A clear illustration is that of blossom formation by alfalfa. Better clusters of blossoms were secured by starting the plants (of the strains used) at a cool temperature and then transferring them to an intermediate temperature (fig. 10, *B*). This change in environment proved more satisfactory in promoting flowering than keeping the plants continuously in either one of the environments. High night temperatures cause the blossoms to abscise (fig 10, *D*).



FIGURE 10.—A change in environment aids alfalfa blossom development (all long-day plants): *A*, Cool; *B*, cool, transferred to an intermediate temperature; *C*, intermediate temperature; *D*, warm (blossoms abscising.)

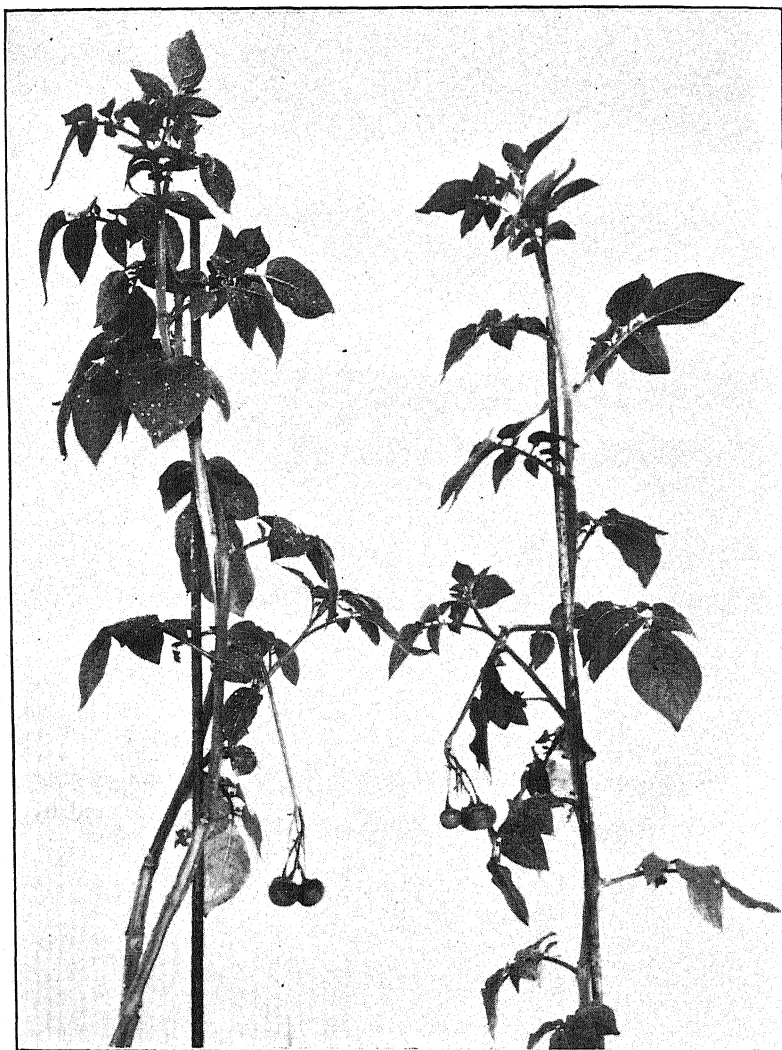


FIGURE 11.—Seed balls produced by Katahdin potato plants grown in cool, long-day environment but started in cool, short-day environment.

Good yields of seed balls of potatoes were produced by plants of the variety Katahdin which had come to an early blossom-bud stage in a cool short-day location and were then transferred to long days (fig. 11). Mature seed balls were not produced by the plants which remained continuously in the short- or the long-day situations.

DISCUSSION

The effect of temperature on the manner in which many plants responded to photoperiod reported here, together with the reactions

recorded last year,⁸ appear to indicate clearly that photoperiod is not always the dominant factor inducing the blossoming of plants. Other cultural factors, such as seed and plant source and the condition of the plant when the treatment is begun, also indicate that the effect of environment is not constant. Photoperiod may be the primary factor for a certain range of temperature, but with many plants it is a contributing and not a controlling factor in the formation of flowers.

A uniform growth of a population may not be evidence that it is a homozygous one. The uniformity may be due merely to the fact that the environment did not induce an expression of the true heterozygous nature of the lot.

From a practical as well as an experimental standpoint, it would seem that more attention should be given to measuring the results secured when plants are shifted from one environment to another at different phases of their development. The most satisfactory growth of many varieties of plants cannot be expected by keeping them under a constant cultural environment throughout the entire period of their development.

SUMMARY

A large number of plants grown in long-day and short-day environments gave very unlike responses to photoperiod at different temperatures.

Corn plants grown in the greenhouse at warm temperatures were normal in appearance.

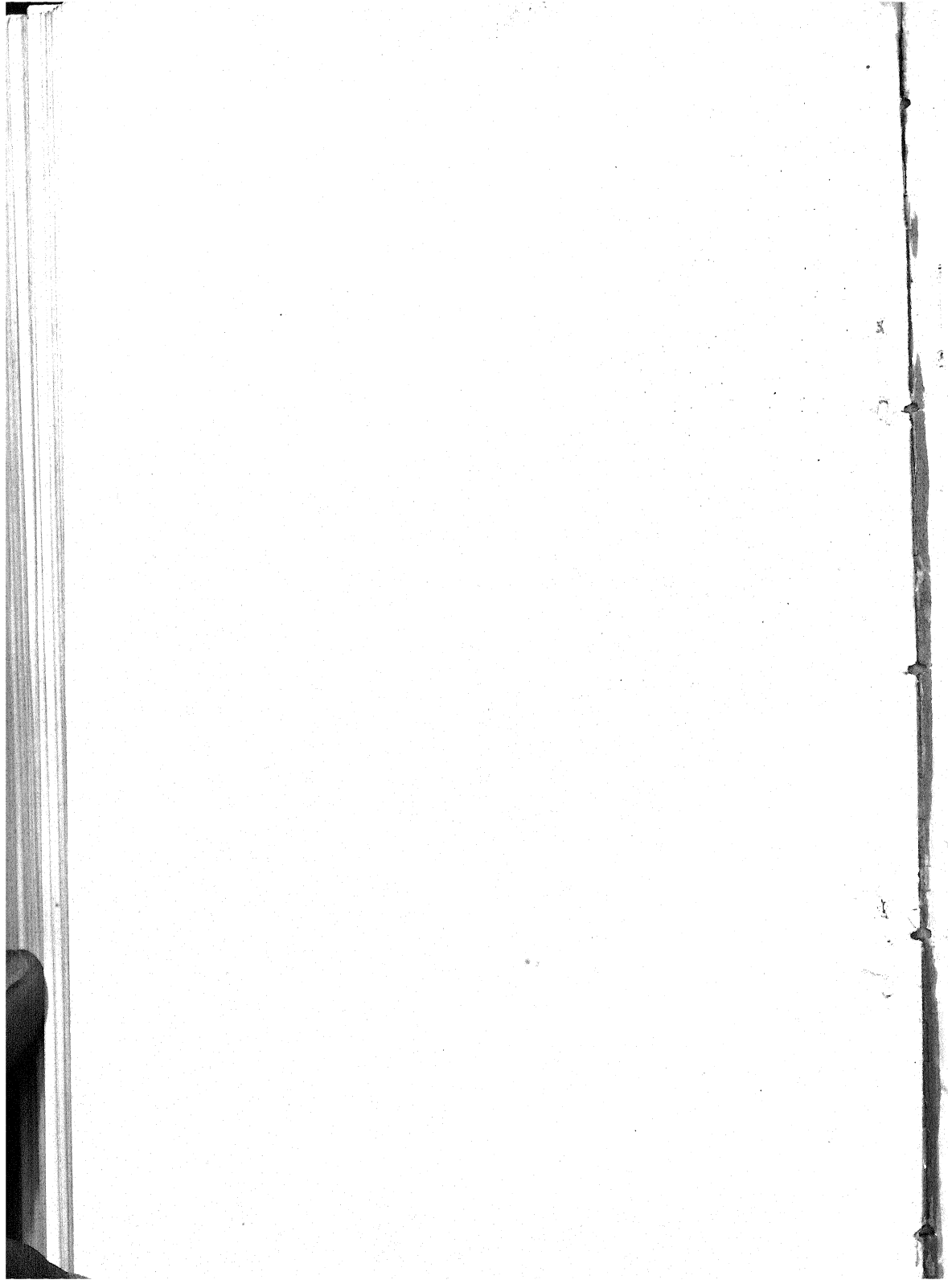
Plants grown from cuttings from flowering plants responded differently to photoperiod than did seedlings.

The variability of a plant population when grown from either seedlings or as clons was largely dependent on the environments in which the plants were grown.

The influence of size of seed on vigor of plant was affected by the environmental conditions under which the plants were grown.

A shift in the environment during the growth of many plants, as alfalfa, appears to aid in securing the most desirable type of development.

⁸ See footnote 2.



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EFFECT OF TEMPERATURE AND MOISTURE ON OVER-WINTERING PUPAE OF THE CORN EARWORM IN THE NORTHEASTERN STATES ¹

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INTRODUCTION

A high percentage of the pupae of the corn earworm (*Heliothis armigera* (Hbn.)) that enter hibernation in the Northeastern States during the fall die before the following spring. An effort has been made for a number of years to determine the primary factors responsible for this mortality. In connection with observations under a variety of soil conditions in Virginia, New Jersey, Connecticut, and Massachusetts, the hibernating quarters of the pupae have been studied with particular reference to the effects of moisture, temperature, and physical condition of the soil. Since the conclusions drawn from cage and field observations are in part based on circumstantial evidence, it seemed advisable to study the relation of the soil conditions to pupal survival experimentally in the laboratory and insectary. The work was done at New Haven, Conn., and Arlington, Va., from 1936 to 1938.

HIBERNATING QUARTERS OF THE INSECT

In the Northeastern States the pupae of the corn earworm spend the hibernation period, from about September to June, or at least 9 months, at the bottom of tunnels in the soil. These tunnels are prepared by the larvae and serve as a means of escape for the moths. They range from 1 to about 8 inches in depth, the majority being from 2 to 4 inches, and extend upward to within about half an inch of the surface. The larva packs the wall of the tunnel and lines it with a network of silk. Thus the pupa is located in a dead-air space, which is an excellent insulator, and rests in a chamber lined with a material that is low in thermal conductivity. The cylindrical form of the tunnel, the packing of the walls, and the silken lining keep the tunnel intact over a long period. Alternate freezing and thawing when the soil is moist, invasion by plant roots, and the burrowing of earthworms are some of the important agencies involved in disrupting these tunnels.

EFFECT OF SOIL TEMPERATURE AND MOISTURE

During the long resting period the pupa is subjected to varying conditions of soil temperature and moisture. When rainfall is heavy and water stands on the soil, the tunnels become flooded, and if the soil freezes while the tunnels are flooded, the pupae may become embedded in ice. Precipitation in the form of snow holds the soil moisture at a minimum during the period of low evaporation and also insulates the soil against low temperatures. In soil containing liberal amounts of organic matter the moisture content is higher and disease organisms are probably more prevalent than in mineral soils.

¹ Received for publication August 5, 1939.

The character of the soil itself often governs the conditions to which the pupae are exposed. Thus, sandy soil is usually better drained, and pupae located in it are less often submerged. A tight clay soil with good surface drainage is often favorable for winter survival. The rate of heat conduction in a soil increases as the amount of soil moisture increases, and ice is an even better conductor than water. A pupa attached to frozen soil by a film of ice will therefore give up its heat much more readily than one having a dry surface and resting on dry soil. The high specific heat of water tends to keep the temperature of the moist soil low.

TEMPERATURE

Observations on exposure in dry surroundings showed that a high percentage of pupae would survive a minimum outside air temperature of about 15° F., but exposure at this temperature in moist surroundings resulted in considerable mortality. With a microvoltmeter it was found that the freezing point of pupae maintained in dry air was about 10°.² The effect of repeated exposures to sub-freezing air temperatures alternated with temperatures above freezing is shown by an experiment summarized in table 1. Ten pupae were exposed to subfreezing air temperatures under dry conditions for 6 periods of 7 to 43 hours and were allowed to warm to 40° in the intervals between exposures. The total time of exposure to freezing temperatures was about 112 hours. It will be noted that these exposures were not fatal.

TABLE 1.—Survival of (10) corn earworm pupae when repeatedly exposed to freezing air temperatures in an insectary

Date	Period of exposure	Temperature range	Pupae surviving	Date	Period of exposure	Temperature range	Pupae surviving
	Hours	° F.	Number		Hours	° F.	Number
Jan. 27-28	24	22-32	10	Feb. 3	8	19-30	10
Jan. 28-30	43	28-33	10	Feb. 4	7	22-31	10
Feb. 2	6	24	10	Feb. 11-12	24	25-31	10

A comparative analysis of 50 hibernating earworm pupae and 100 hibernating European corn borer (*Pyrausta nubilalis* (Hbn.)) larvae on December 11, 1936, showed that the former contained 67.5 percent of moisture and 13.7 percent of fat, whereas the latter contained 55.4 percent of moisture and 20.8 percent of fat. The higher moisture and lower fat content and the exposure to more adverse moisture conditions probably explain why the corn earworm pupae are more susceptible to low temperatures than are corn borer larvae, although in their respective hibernating habitats the earworm pupae are not subjected to such low temperatures as are the corn borer larvae.

MOISTURE

It has long been known that dry soil is favorable for survival of hibernating pupae. When placed in the atmosphere of an ordinary room, or in cardboard or metal boxes, a large proportion of the pupae survive the winter, even though the environment remains warm.

² Determined in the laboratory of Roger B. Friend, of the Connecticut Agricultural Experiment Station during the winter of 1937.

The slight loss of their original moisture content under these conditions seems to have little effect on their ability to emerge as moths.

In September 1936, 25 larvae were allowed to dig hibernating quarters in a box containing about 1 cubic foot of dry soil. This box was left indoors. Between July 7 and August 16, 1937, 14 individuals emerged as moths, 9 individuals died as pupae, and 1 died as a larva. In another experiment just a year later 100 larvae were allowed to pupate in 2-ounce tin salve boxes and were kept at room temperature throughout the winter. On April 18, 1938, or 6 months later, 62 pupae were alive.

On November 11, 1937, 25 pupae recovered from outside hibernation cages that had been established about September 15 were placed in an open cardboard box. These pupae were retained in a heated office and were weighed daily for 1 month. On November 12 the average weight was 0.541 gm., and by December 11 it had decreased to 0.486 gm. Thus, in a single month of exposure in a heated room these pupae lost 10 percent of their weight. Some weight was lost each day, but the rate of desiccation was highest during the first 5 days of the experiment. Twenty-four individuals emerged as moths between December 13 and April 18. The ability of the pupae to withstand loss of moisture allows the insect to survive dry conditions during both hibernation and the growing season.

The pupa is covered with an oily material, which permits it to shed water. When the humidity of the burrow is high, small beads of moisture appear on the surface of the pupa, especially when the surrounding soil is warm. Normal soil moisture (without flooding) does not seem to be injurious, but probably is advantageous in maintaining a moisture balance.

In contrast to a loss of 10 percent of their weight in 1 month by pupae kept under dry conditions in a heated room, another lot of 25 pupae kept between moist paper towels in a cool room lost only 1.7 percent of their weight in over 2 months. On November 25 these pupae weighed on an average 0.529 gm. and on January 29, 0.520 gm. On May 10, 22 of these pupae were alive. This was a higher rate of survival than that obtained in any other lot of pupae that the writers have carried through hibernation.

In their burrows the pupae usually encounter a moist environment, but they are subjected to periods of drought and to periods of submergence during or following heavy rainfall. During such dry or wet periods they lose or gain weight. Changes in weight of pupae subjected to different successive degrees of moisture are shown in the experiment summarized in table 2. When exposed to dry air (simulating drought conditions) loss of weight was rapid; when in contact with moist earth (simulating the usual pupal environment) a slight loss of weight occurred; when submerged in artificial burrows (simulating the conditions during heavy rainfall) pupae gained slightly in weight. Under the first two conditions all the pupae survived, but the last condition was hazardous and caused a heavy mortality.

When hibernation cages are examined in the spring, pupae are often found which appear normal in all respects except that they are immobile. When exposed to room temperatures, some of these individuals are revived and emerge as apparently normal moths. In the

experiments that have been described similar individuals were encountered. The observations indicate that the causes of death in the hibernation cages and in the experiments are similar and that considerable pupal mortality is caused by the smothering effect of water, some of which may be absorbed by the tracheal system.

TABLE 2.—Average changes in weight of 2 lots of 15 corn earworm pupae subjected to different successive conditions of moisture

Lot	Environment	Period of exposure	Average weight of 1 pupa		Gain (+) or loss (−) in weight	Pupae surviving
			At beginning	At end		
			Gram	Gram	Percent	Number
1	Dry air of room.....	Dec. 9-14.....	0.550	0.521	−5.3	15
	Flooded burrows at room temperature.....	Dec. 14-20.....	.521	.526	+1.0	7
	Dry air of room.....	Dec. 9-14.....	.526	.484	−8.0	15
2	Buried in moist earth at room temperature.....	Dec. 14-20.....	.484	.482	−.4	15
	Dry air of room.....	Dec. 20-27.....	.482	.466	−3.3	15
	Submerged in burrows at room temperature.....	Dec. 27-Jan. 3.....	.466	.472	+1.3	1

EFFECT OF SUBMERGENCE IN WATER

An observation in soil saturated with water at Charlottesville, Va., in August 1932, revealed the pupae floating in the top of the tunnels. Apparently such pupae recede to the bottom of the tunnels with the water level or work back to their normal position by their wriggling movements. Experiments in the laboratory with pupae placed in glass tubes (of a diameter similar to that of the hibernating burrows) partly filled with distilled water gave information on the proportion of pupae that would float, how long they would float, and the effect of floating on survival. The results of such an experiment are summarized in table 3. At the end of the period of treatment 62.5 percent of the pupae were floating. There was variation in the position of some of the pupae from day to day. Thus, 57.5 percent floated during the entire period of observations, 20 percent were submerged for the entire period, 17.5 percent of those that floated at first sank later, and 5.0 percent changed their status more than once, that is, they floated at first, then sank, and later floated again. These data show that, if pupae floated, some survived for as long as 20 days, whereas if they were submerged without floating they died within less than 10 days.

TABLE 3.—Effect of submergence of corn earworm pupae for different periods in artificial burrows partly filled with water ¹

Duration of immersion (days)	Pupae floating at end of period	Pupae submerged at end of period	Survival of pupae that floated	Survival of pupae that sank
	Number	Number	Number	Number
5.....	4	6	2	4
10.....	7	3	5	0
15.....	5	5	2	0
20.....	9	1	4	0
Total.....	25	15	-----	-----

¹ These experiments were conducted in an unheated basement room, the air temperature of which was about 60° F. 10 pupae were immersed for each period.

Of a series of 25 pupae submerged at 75° F. in lots of 5 for intervals of from 2 to 10 days, 20 percent survived. In a similar series submerged for like periods at 40°, 84 percent survived. The much higher mortality in water at 75° was due, no doubt, to the higher rate of metabolism with insufficient oxygen. This is probably an important factor in mortality of hibernating pupae under high soil-moisture conditions in the spring when higher soil temperatures prevail.

The importance of high soil moisture in the summer mortality of the soil stages of the earworm is indicated in the results of an experiment at Arlington, Va., begun August 20, 1937. A total of 312 ears infested with larvae, mostly in the fifth and sixth instars, were impaled in cages. Most of the larvae entered the soil during the following 10 days, which was a period of heavy rainfall. On September 15 these cages were examined, with the following results: Living pupae, 122; dead pupae, 129; pupal exuviae, 24; total mortality, 46.9 percent.

General observations in hibernation cages confirm these results. It seems, therefore, that pupae may survive flooding for short periods following heavy rainfall, and that the rate of mortality increases as the temperature increases. The ability of the pupae to float in their burrows doubtless accounts for the high percentage of survival that is often encountered after rather prolonged wet periods.

COMPARATIVE WINTER MORTALITY OF PUPAE RESTING ON A DRY AND ON A WET MEDIUM

During the winters of 1936-37 and 1937-38 two lots of pupae, one lot resting on a dry medium and the other on a moist medium, were exposed to outdoor temperatures at Arlington. In 1936-37 another lot was exposed on moist sand. These pupae had been collected from soil in hibernation cages or field plots about the middle of November and were placed in 2-ounce salve boxes containing about three-eighths of an inch of sand or loam. The pupae used in 1936-37 matured on sweet corn (*Zea mays* var. *rugosa*). In 1937-38 some of the pupae had been collected at Moorestown, N. J., and at Marietta, Ohio, on Evergreen sweet corn; others had been collected at Arlington, the larvae having matured on field corn (*Z. mays*) in the dough stage. No moisture was added to the boxes containing the dry sand or soil, but moisture was added occasionally during the winter to the boxes started with moist sand or soil. Two holes of uniform size were punched in the lid of each box to provide ventilation.

The survival of these pupae throughout the winter is given in tables 4 and 5. The relative survival on moist media and also on dry media was similar in both years; however, the actual survival on the dry media was nearly twice as high at the end of the second-year period as at the end of the first-year period. A great reduction in surviving pupae occurred during the early part of the winter of 1936-37, when there were rapid drops to below-freezing temperatures. It is believed that these sudden exposures account for most of the difference in survival between the 2 years.

The effect of differences in the thermal conductivity of dry and moist environments on the pupal mortality was strikingly brought out in the 1936-37 experiments on moist and dry soil. The pupae

had been collected in the field on November 23 and stored at about 70° F. until November 30 in order to eliminate as much as possible insects injured in handling. The temperature at the beginning of the exposure was 40°. It dropped to below freezing after a few hours, reaching a minimum of 15° for about 2 hours early the next day. On December 1 the temperature was above freezing for about 5 hours, reaching a maximum of 42°, and dropped to a minimum of 29° early on December 2. An examination on December 2 showed a mortality of 56.9 percent on moist soil and 15.7 percent on dry soil. Since the internal moisture of the pupae in this instance could hardly have changed enough to affect their freezing point, it is evident that the pupae resting on moist soil gave up their heat more readily than those on dry soil.

TABLE 4.—*Survival of corn earworm pupae when resting on moist sand and on moist and dry soil in an insectary at Arlington, Va., throughout the winter of 1936-37*

Observation period ¹ beginning—	Minimum temperature	Days below freezing	Survival of pupae at end of period			Observation period ¹ beginning—	Minimum temperature	Days below freezing	Survival of pupae at end of period		
			100 pupae on moist sand	51 pupae on moist soil	51 pupae on dry soil				100 pupae on moist sand	51 pupae on moist soil	51 pupae on dry soil
	° F.		Percent	Percent	Percent		° F.		Percent	Percent	Percent
Nov. 24....	19	3	30.0			Jan. 23....	30	2	.0	3.9	50.9
Nov. 30....	15	2	13.0	43.1	84.3	Jan. 30....	21.5	5	.0	.0	50.9
Dec. 2....	27	4	13.0	39.2	76.5	Feb. 6....	20	5	.0	.0	49.0
Dec. 7....	22.5	2	9.0	7.9	74.5	Feb. 13....	22	5	.0	.0	49.0
Dec. 11....	27	4	8.0	7.9	74.5	Feb. 20....	19	6	.0	.0	33.3
Dec. 16....	21	6	6.0	5.9	58.8	Feb. 27....	19	3	.0	.0	27.4
Dec. 24....	27	3	4.0	3.9	54.9	Mar. 6....	22	5	.0	.0	27.4
Jan. 4....	25	1	4.0	3.9	52.9	Mar. 13....	28	6	.0	.0	27.4
Jan. 9....	35	0	3.0	3.9	50.9	Mar. 20....	26	2	.0	.0	27.4
Jan. 16....	33.5	0	3.0	3.9	50.9	Mar. 27....	31	2	.0	.0	27.4

¹ Each observation period begins at the conclusion of the next preceding period.

TABLE 5.—*Weekly survival of corn earworm pupae when resting on moist and dry sand in an insectary at Arlington, Va., throughout the winter of 1937-38*

Week beginning—	Temperature		Days below freezing	Survival of pupae at end of period		Week beginning—	Temperature		Days below freezing	Survival of pupae at end of period	
	Mean minimum	Minimum		152 pupae on moist sand	148 pupae on dry sand		Mean minimum	Minimum		152 pupae on moist sand	148 pupae on dry sand
	° F.	° F.		Percent	Percent		° F.	° F.		Percent	Percent
Nov. 13....	40.6	33	0	100.0	100.0	Jan. 22....	27.0	15	5	5.9	56.8
Nov. 20....	24.6	20	5	71.7	96.7	Jan. 29....	26.4	15	3	3.3	54.1
Nov. 27....	35.4	26	2	71.1	96.7	Feb. 5....	31.9	28	3	3.3	54.1
Dec. 4....	23.0	16	5	52.0	90.5	Feb. 12....	32.6	23	3	3.3	52.7
Dec. 11....	22.4	14	5	18.4	81.8	Feb. 19....	32.4	27	3	3.3	52.7
Dec. 18....	34.0	27	2	17.8	78.4	Feb. 26....	25.4	19	5	1.3	52.0
Dec. 25....	30.4	22	4	17.8	77.7	Mar. 5....	30.3	21	3	1.3	52.0
Jan. 1....	30.0	24	5	17.8	76.4	Mar. 12....	39.7	32	1	1.3	52.0
Jan. 8....	26.1	18	6	13.8	75.0	Mar. 19....	45.3	34	0	1.3	52.0
Jan. 15....	23.6	14	6	8.6	66.9	Mar. 26....	42.3	31	1	1.3	52.0

The rate of pupal survival in relation to temperature and moisture for the winter of 1937-38 is also shown graphically in figure 1. The heaviest mortality occurred before December 18 among the individuals on moist sand. The minimum temperature recorded during this period was 14° F. Temperatures of 15° or below were recorded on 4 days during December and January. December had the lowest mean temperature of the winter months. Evidently some individuals were more susceptible to freezing temperatures than others, particularly among the group on moist sand. After December 18 the remaining pupae on wet sand suffered less mortality than those on dry sand. That pupae on dry sand were injured by low temperatures was evident from a mottled brown to dark-brown appearance of

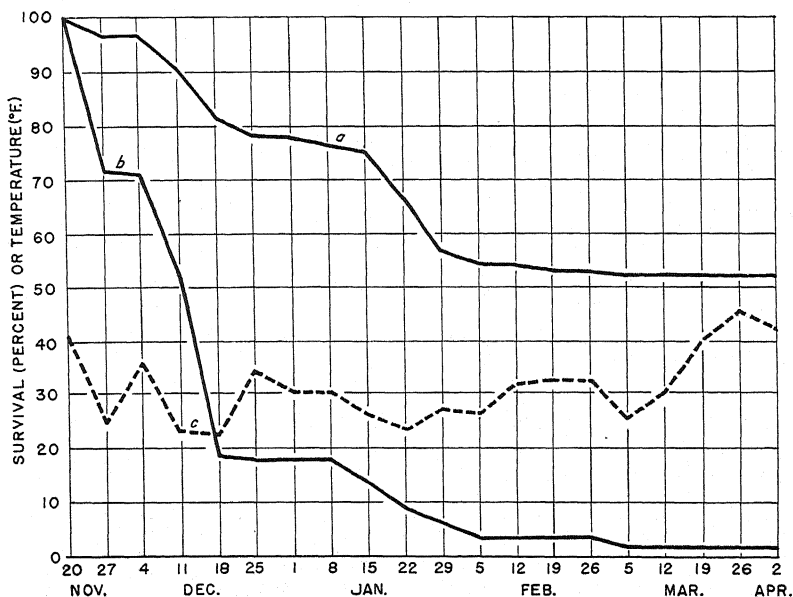


FIGURE 1.—Weekly survival of hibernating pupae in salve boxes on dry and on moist sand, exposed to outside temperatures during the winter of 1937-38, Arlington, Va.: *a*, Survival on dry sand; *b*, survival on moist sand; *c*, mean minimum temperature to which the pupae were exposed.

some individuals. Such pupae usually died within a few weeks after receiving this injury.

Of the 14 pupae that survived the winter of 1936-37, 12 produced moths and 2 died between May 31 and July 13. In 1938, 69 moths emerged and 10 pupae died between May 24 and July 8 from a total of 79 pupae that were surviving on April 2. In general, the data are significant in showing that hibernating pupae of the earworm are able to withstand rather severe soil temperatures, particularly when the soil is dry.

The only pupae that survived on moist sand or soil during the two winters developed from larvae that matured on field corn in the dough stage. There was very little difference in mortality of pupae resting on a dry medium whether sweet or field corn had been the host, nor

was there any significant difference among the lots from the different collection points.

The results are similar to those observed in hibernation experiments for a number of years, particularly in the winter of 1935-36, when an unusually high mortality followed heavy rains in January and a rapid drop in temperature which continued at a low point for approximately 1 month. During the early part of this period there was little protection from snow cover, and frost penetrated the wet soil to a depth of about 2½ feet. This condition was followed by snowfall, which acted as a good insulator for holding the soil temperature at or below the freezing point. Under these conditions the temperature in clay containing some fine sand and much organic matter reached a minimum of 22.5° F. at a depth of 4 inches.

EFFECT OF AVAILABILITY OF AIR

Although the vital processes in animals are at a low ebb during hibernation, a certain amount of respiration is necessary. During the

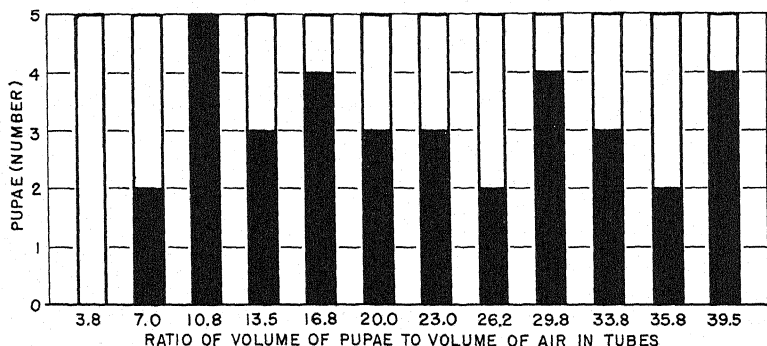


FIGURE 2.—Survival of pupae after 6 months at 40° F. in tubes in which the volume of available air ranged from 3.8 to 39.5 times the volume of the pupae. Each column represents five pupae in individual tubes having the same volume of air, the solid portions showing number of pupae alive at the end of the experiments.

winter, when the ground is frozen for several weeks at a time, the pupae of the corn earworm are often sealed in their burrows with ice. The question arises, Do the pupae smother under such conditions?

A total of 60 pupae were placed individually in glass tubes of a diameter similar to that of the insects' hibernating tunnels ($\frac{3}{8}$ inch) and of 12 different lengths ranging from 1½ to 12½ inches. The ends of each tube were closed by cork stoppers cut flush and then sealed with waterproof glue. The volume of air in these tubes ranged from 1.9 to 16.2 cc. It was found that the average displacement of air by a pupa was about 0.4 cc. The volume of air in the tubes therefore ranged from 3.8 to 39.5 times the volume of the pupa. The tubes were stored in a cold room at about 40° F. from October 9, 1936, until May 5, 1937, a period of 208 days. At the end of the period 35 pupae, or 58 percent, were found to be alive (fig. 2). Except for the individuals confined in the smallest volume of air, mortality was probably due to causes other than lack of air.

Of 120 pupae in individual unsealed salve boxes under similar temperature conditions and for a similar period, 44 percent were alive on April 7, 1937.

These experiments indicate that, in the tunnels of average length, it is unlikely that pupae die from lack of air during hibernation, even when the soil is frozen solidly about the burrows for prolonged periods.

EFFECT OF BEING EMBEDDED IN ICE

Following heavy rainfall and a sudden drop to subfreezing temperatures pupae may become partly or wholly embedded in ice. This condition was simulated in a refrigerator. Ten pupae were taken from storage at 40° F. and placed at the bottom of small paper cups filled with water. The cups were then placed in a tray in the refrigerator away from contact with the freezing unit. The air temperature at the location of the pupae was about 26° F., and an exposure of at least 8 hours was necessary to freeze the water solidly. After 7 hours the water had frozen except at the center of the cups. All the pupae survived this treatment. When exposed for 18 hours 1 of 10 pupae survived, and when exposed for 24 hours all the pupae died. In the last 2 instances the pupae had been embedded solidly in ice for approximately 10 and 16 hours, respectively.

In January, 10 pupae in similar cups of water were exposed for 24 hours outdoors at temperatures ranging from 22° to 32° F. At the end of this period they were embedded solidly in ice. Only 1 pupa survived this treatment. On February 2, 10 pupae were exposed under similar conditions for 6 hours at 24°, and although they were embedded solidly in ice at the end of the period, 8 survived. Another series of 10 pupae were exposed similarly on February 4-5 for 24 hours at a temperature of 30°-32° most of the time. At the end of the period the water in the center of the cups had not frozen. All these pupae survived.

EFFECT OF EARTHWORM ACTIVITY AND OF BEING EMBEDDED IN SOIL

In localities where the soil does not freeze repeatedly and when they are not disrupted by the soil fauna or by plant roots, the pupal tunnels, because of their firm construction, are often found to be in perfect condition as much as a year after being formed. In the Northeastern States several factors cause disruption and filling in of the tunnels. Important among these factors are heaving and settling of the soil due to alternate freezing and thawing. Earthworms disrupt a high percentage of the tunnels in soils favorable to them, especially during wet periods when their activity is concentrated near the surface.

The probable effect on emergence of moths from tunnels that have been partly or wholly filled with the droppings of earthworms has been pointed out by Phillips and Barber.³ The present writers' observations on hibernation cages located in clay-sand soil high in organic matter at Arlington from 1934 to 1937 indicated that a considerable mortality among hibernating pupae was caused directly or indirectly by intense earthworm activity. In 6 cages, each stocked

³ PHILLIPS, W. J., and BARBER, GEORGE W. A STUDY OF HIBERNATION OF THE CORN EARWORM IN VIRGINIA. Va. Agr. Expt. Sta. Tech. Bul. 40, 24 pp., illus. 1929.

in the fall with 100 full-grown larvae, there was no survival in the 3 years of the experiment. By the middle of April the soil was perforated with earthworm tunnels and the tunnels of the earworm had been practically obliterated. The pupae had collapsed and disintegrated to such an extent that only the exoskeletons remained. This rapid disintegration of pupae embedded in the droppings was probably brought about by the nitrogenous environment. In 6 similar cages containing a sandy loam in which earthworms were much less active the pupal survival in the 3-year period was 9.8 percent.

Since soils favorable for earthworms are rich in organic matter and have a high moisture-holding capacity, which in itself has been shown to be unfavorable for survival, it is difficult to determine under field conditions to what extent the droppings of earthworms are directly toxic to the pupae.

The mild winter of 1936-37 at Arlington practically eliminated freezing soil temperatures as a factor in winter mortality. The soil moisture was high, and earthworm activity was concentrated near the surface for a considerable period. In table 6 are given the results of examinations in a field plot in which there was a heavy population of earthworms. There was little, if any, pupal mortality on November 23 that could be attributed to earthworm activity in the tunnels. By March 22 the survival had dropped to 38.9 percent, and the mortality continued at about the same rate from March 22 to April 19.

TABLE 6.—*Survival of hibernating pupae of the corn earworm in a field plot having a high earthworm population, Arlington, Va., 1936-37*

Date	Pupae recovered	Living pupae		Dead pupae		Survival
		Free or partly embedded	Embedded	Free or partly embedded	Embedded	
	Number	Number	Number	Number	Number	Percent
Nov. 23.....	184	170	0	14	0	92.4
Mar. 22.....	95	37	0	25	33	38.9
Apr. 19.....	101	24	0	14	63	23.8

The heavy rainfall during the winter months undoubtedly accounted for most of the mortality, since of the 58 dead pupae recovered on March 22, 25 were not embedded. A decrease in the number of dead pupae not embedded on April 19 indicates that many of the embedded specimens had died prior to becoming embedded. All the living pupae were found in tunnels, in which they were permitted some degree of movement. Movement of the abdomen appears to be essential for the insect to survive embedding in moist soil for any length of time. In the spring 8 living pupae were found with the thoracic region rather firmly embedded but free to move the abdominal segments.

In experimental shallow embedding on May 8, 1937, 10 pupae were covered with mud and allowed to dry on a paper tray. Owing to the oily nature of their integument, they freed themselves of much of the mud by wriggling. From these pupae 8 moths emerged, but in 2 instances the mud adhered too closely for the moths to escape from the pupal cases. From 10 pupae covered with a layer of mud three-fourths of an inch thick, which later dried and hardened, 1 pupa

was alive on July 1 and 9 had become moths but were unable to free themselves from the pupal cases. Of 10 pupae covered with a $\frac{3}{4}$ -inch layer of moist packed soil, all individuals wriggled to the surface and emerged as moths. There was complete emergence from 10 pupae placed on a paper tray out of contact with soil.

The data presented substantiate the previous indications that soil moisture is an important factor contributing to the mortality of hibernating pupae. There is, however, no conclusive evidence that the biotic and chemical conditions brought about and associated with intense activity of earthworms in the soil at hibernating levels are directly toxic to the pupae. Earthworm activity at and above the hibernating level of the pupa coincides with high soil moisture, and obstructions in the tunnels under such conditions subject pupae to longer than normal submergence.

EFFECT OF THERMAL CONDUCTIVITY IN SOILS

It has been shown that pupae succumb in much greater numbers when exposed to the same temperature while resting on a moist medium than on a dry medium. When the conditions in soils varying in moisture content are considered, it is very evident that the transmission of heat from the pupae is an important factor in mortality. As water is transformed from the liquid to the solid state, the thermal conductivity is more than quadrupled. The thermal conductivities of various substances involved, expressed in calories, are as follows: Air 0.000056, water 0.0012, ice 0.005, compact snow 0.00051, dry sand 0.00093, dry soil 0.00033, and silk (similar to chitin in nature) 0.000095⁴. When the insulating efficiency of dry air, dry soil, and a dry exoskeleton is considered in addition to the reduced water content of pupae hibernating under dry conditions, the primary reason for a higher survival in such an environment is quite obvious. Pupae resting in wet soil, a good conductor of heat, have a moist exoskeleton and are practically fused to the soil when it freezes, thus allowing heat to flow readily from their bodies.

Experiments on hibernation in this area in a variety of soils have shown that the rate of survival is highest in well-drained soil, usually sand. The reason for this may be inferred from a study by Bouyoucos⁵ of soil temperatures under laboratory and field conditions. He found that the specific heat of gravel, sand, loam, clay, and peat soils did not differ materially, but that under field conditions their water-holding capacity differed greatly, and that, because of the high specific heat of water, the differences in water content accounted for the differences in soil temperature. The temperatures of these soils varied but little during the winter, the gravel and the sand being slightly colder. The lower water capacity of the gravel and sand, the greater radiation of heat in these types of soil, and the higher thermal conductivity resulted in their thawing out earlier and being more responsive to air temperatures in the spring. Organic matter increased the water-holding capacity of the soil, peat holding about

⁴ These figures were taken from a compilation in the following: CHEMICAL RUBBER COMPANY. *HANDBOOK OF CHEMISTRY AND PHYSICS, A READY-REFERENCE BOOK OF CHEMICAL AND PHYSICAL DATA.* Ed. 20, 1951 pp., illus. Cleveland, Ohio. 1935. See pp. 1301-1302.

⁵ BOUYOUCOS, GEORGE J. *AN INVESTIGATION OF SOIL TEMPERATURE AND SOME OF THE MOST IMPORTANT FACTORS INFLUENCING IT.* Mich. Agr. Expt. Sta. Tech. Bul. 17, 196 pp., illus. 1913.

25 times as much water as the mineral soils sand and gravel. The factors thus shown to be characteristic of gravelly and sandy soils—i. e., more rapid warming up in the spring and lower water content, together with the good drainage which is usually characteristic of mineral soils like sand and gravel—are no doubt responsible for the more successful hibernation found in such soils.

It was shown in table 2 that in room atmosphere the pupae lose moisture rather rapidly, but that moisture is again absorbed upon subsequent exposure. The moisture is perhaps absorbed by the sclerotic exoskeleton and the tracheal system. The freezing of this absorbed moisture is apparently injurious to the tissues affected. This type of injury was particularly apparent in the integument of pupae resting on dry sand. When such pupae were exposed to temperatures below 20° F., some individuals had a mottled appearance, the dark areas discernible in the integument resembling typical frost-bite. Pupae so injured usually died within a few weeks. Pupae killed by freezing while resting on moist sand were somewhat distended and rather uniformly dark brown, eventually turning almost black.

SUMMARY

In order better to interpret observations in the field, laboratory and insectary experiments have been conducted at New Haven, Conn., and Arlington, Va., on the effect of moisture and temperature on hibernating pupae of the corn earworm (*Heliothis armigera* (Hbn.)).

The pupa hibernates in the soil from about September to June at the base of an upward-sloping cylindrical tunnel, usually at a depth of from 2 to 4 inches. The wall of the tunnel is packed and lined with silk, which helps to hold it intact for long periods of time. Heaving of the soil caused by alternate freezing and thawing and invasion of plant roots and earthworms are important agencies in disrupting the tunnels during the hibernating period, causing pupae to become embedded in the soil and die, and also interfering with exuviation and emergence. Well-drained soils, particularly sandy ones, provide conditions favorable for survival.

The freezing point of pupae maintained in dry air was found by the microvoltmeter method to be about 10° F. Analyses of some hibernating pupae, made in December, showed 13.7 percent of fat and 67.5 percent of water as compared with 20.8 percent of fat and 55.4 percent of water in hibernating European corn borer larvae.

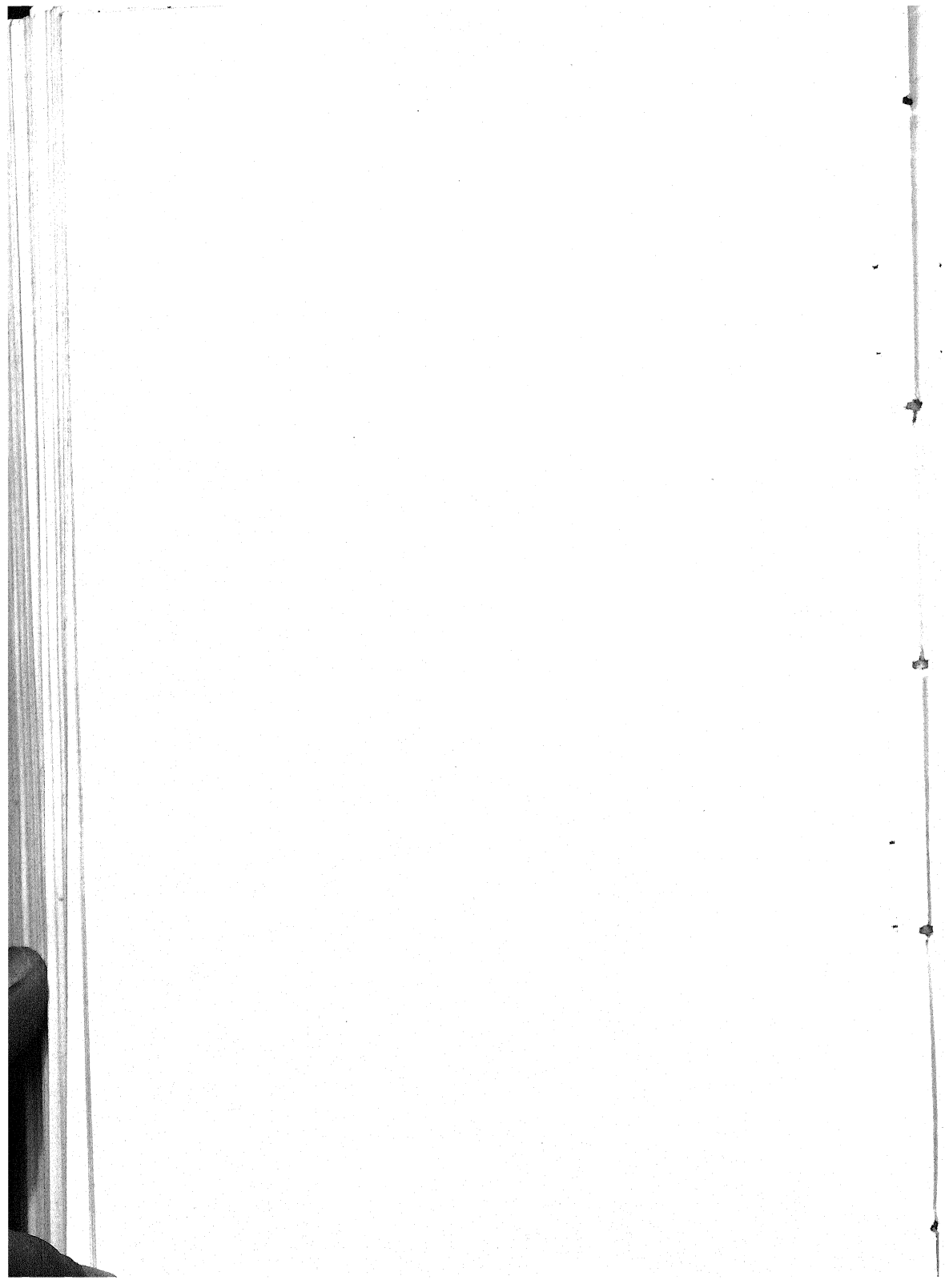
Hibernation indoors in the atmosphere or in salve boxes in a heated room results in but little mortality and has little effect on the ability of moths to emerge. Pupae exposed 1 month in a heated room lost 10 percent in weight in contrast with 1.7 percent for pupae kept between moist paper towels in a cool room. When pupae are submerged in water after losing weight in dry air they gain slightly in weight. This gain seems to be due to absorption of water by the exoskeleton, the tracheal system, or both.

The pupae are able to float in flooded tunnels. In the laboratory they were observed to float, sink, and then float again. Individuals that did not float died within a short time. The ability to float in flooded tunnels permits survival in periods of heavy rainfall. The mortality among submerged pupae increases with a rise of tempera-

ture. At 75° F. 20 percent survived submergence for 10 days, in contrast to 84 percent at 40°.

During the winter of 1936-37, 27.4 percent of the hibernating pupae exposed in an outdoor insectary at Arlington, Va., survived in 2-ounce salve boxes on dry soil, whereas there was no survival on moist soil. In the winter of 1937-38, under similar conditions, 52 percent survived on dry sand and 1.3 percent on moist sand. The minimum temperature during these two winters was 14° F. The higher rate of survival on dry soil is attributed to the better insulating efficiency of a dry environment and of a dry pupal exoskeleton and to the lower water content of pupae hibernating under dry conditions. Pupae resting on wet soil are more susceptible to below-freezing temperatures because of a higher thermal conductivity of moist soil, more body water, and a moist exoskeleton, which practically becomes fused to the soil when it freezes, allowing the body heat to be given up readily.

In sealed tubes ranging from 1½ to 12½ inches in length there was survival in all but the shortest at the end of 208 days of exposure at 40° F. Of 10 individuals, 1 survived being frozen in ice for 18 hours. An exposure of 24 hours was fatal.



THE *D Rs P* LINKAGE GROUP IN SORGHUM¹

By J. C. STEPHENS, associate agronomist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and J. R. QUINBY, superintendent, Substation No. 12, Texas Agricultural Experiment Station and agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

This paper reports a linkage group of three factors in sorghum (*Sorghum vulgare* Pers.) and the independence of this group from several other factors that have been reported previously by the writers or by other workers. The linked factors are those for dry and juicy stalks (*Dd*), red and green seedling stems (*Rrsr*), and purplish and brownish plant color (*Pp*). These linkages were studied in the coupling phase.

REVIEW OF LITERATURE

Rangaswami Ayyangar (6)² reported that Benson and Subba Rao in 1906 recorded the observation that dry-stalked sorghums have a white midrib and that juicy-stalked varieties have a dull midrib. In 1916 Hilson (2) reported a single-factor difference between dry and juicy stalks, with dry stalks dominant to juicy. Swanson and Parker (14) assigned the symbols *D* and *d* to the factors for dry and juicy stalks, respectively, and illustrated the differences in midrib color. These authors found a higher percentage of smutted plants in rows homozygous for juicy stalks than in rows with dry-stalked plants. It was suggested, however, that the association may have been between smut susceptibility and sweetness rather than smut susceptibility and juiciness. Rangaswami Ayyangar et al. (9) reported that juiciness and sweetness are inherited independently. Rangaswami Ayyangar and Kunhi Koran Nambiar (7) found independent segregation of factors for juiciness and a character called mechanical tissue brown.

In 1930 Reed (11) described and illustrated the contrasting characters red and green seedling stems. He found seedling stem color to be monogenic in inheritance, with red stems dominant to green. Karper and Conner (3) reported linkage between seedling stem color and a seedling albino, with 41.34 percent crossing over. A two-factor segregation for seedling stem color was reported by Woodworth (15) in a cross between shallu and Black Spanish broomcorn.

Rangaswami Ayyangar et al. described and illustrated in color (10, *pl. XLII*) the contrasting plant colors purple and brown. Purple was dominant to brown, and a 3:1 segregation was obtained in *F*₂. The symbols *P* and *p*, respectively, were assigned to the factor pair for these plant colors. Rangaswami Ayyangar et al. (8) reported a

¹ Received for publication June 27, 1939. Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Texas Agricultural Experiment Station at Substation No. 12, Chillicothe, Tex. Technical Series No. 515, Texas Agricultural Experiment Station. Assistance in the preparation of these data was furnished by the personnel of the Works Progress Administration; official project No. 655-66-3-359.

² Italic numbers in parentheses refer to Literature Cited, p. 730.

linkage between the factor pair determining plant color and that for juiciness of stalk, with 30 percent crossing over in the F_2 repulsion phase.

With the exception of the male sterile character, the literature for all characters mentioned in this paper has been reviewed recently in connection with a report on the *Q B Gs* linkage group (13).

A male sterile character, recessive to the normal condition and characterized by incompletely developed anthers, was described in 1937 (12). This factor pair was given the symbols *Ms* and *ms*.

DESCRIPTION OF CHARACTERS

JUICINESS

In some varieties of sorghum the leaf midribs have a chalky-white appearance. If a mature stalk of one of these varieties is split the pith is found to be relatively dry. In other varieties midribs have a dull or opaque appearance, and the stalks of these varieties contain appreciable amounts of juice. That the dull appearance of the midrib in juicy varieties is due to juice may be shown by breaking a leaf and pressing juice out of the midrib, after which it has much the same chalky appearance as midribs of dry varieties. Little or no juice can be pressed from the midribs of dry varieties. The contrasts in midrib color are well shown in illustrations by Swanson and Parker (14) and by Rangaswami Ayyangar et al. (8).

Seedlings and new leaves of all sorghums have dull midribs. The white midrib of dry varieties starts as a narrow streak in the center and gradually spreads toward the margins until the whole midrib is white. Juicy varieties usually have a white streak through the center of each midrib before the plants are mature. However, the white streak begins to show much later in the plant's development than in the case of dry varieties, and the margins of the midribs are always dull. Midrib appearance (color) is generally used to separate dry and juicy plants in segregating populations, and usually the separations can be made readily.

SEEDLING STEM COLOR

Many varieties of sorghum, including broomcorn, Sudan grass, most of the milos and kaoliangs, and many of the sorgos, have red seedling stems. Other varieties, including the Blackhull kafirs and most of the Orange group of sorgos, have green seedling stems. Frequently the contrast between red and green can be observed as soon as the coleoptiles appear at the surface of the ground, but the red color becomes more intense after exposure to light for a few days. In red seedlings the color is most intense in the coleoptile, but sometimes the sheath and under side of the blade of the first to third leaves also are colored red wholly or in part. Below the surface of the soil or in the absence of light, both red and green seedlings are usually white. The contrast between red and green seedling stems was clearly illustrated by Reed (11, *figs. 24 and 25*). Color varies somewhat in intensity and shade between varieties; in Sumac sorgo, for instance, the color is dark purplish red, whereas in milo it is salmon red. Within the experience of the writers, however, the presence or absence of red seedling stem color is due to a single-factor pair, and such variations in intensity or shade as exist between varieties are

probably caused by unidentified modifiers. The F_2 generation of the shallu \times Black Spanish broomcorn cross, wherein Woodworth (15) reported a two-factor segregation, has been grown at Chillicothe, Tex., only once, and environmental conditions were such that phenotypes could not be separated satisfactorily.

Under field conditions, environment influences expression of seedling stem color to such an extent that it is often impossible to separate classes accurately in a segregating population. The exact environmental factors that are responsible for failure of full development of color have not been determined, but trouble in separating phenotypes usually occurs if seedlings emerge when soil is relatively dry and temperature high, particularly if wind is high also. Under such conditions, the red color of red seedlings fails to develop its usual intensity and the green color of green seedlings becomes dark or somewhat gray, and the two types are hard to separate. It is then necessary to grow seedlings of the next generation to determine seedling stem color.

PLANT COLOR

Rangaswami Ayyangar et al. (10) have reported that nearly all sorghums except one group—represented in the United States by the variety shallu—have purple pigmentation. The purple pigmentation is conspicuous in leaf sheaths and glumes. In the absence of purple pigmentation, tissues are brownish. Purple is a simple character dominant to brown, and the symbols *P* and *p*, respectively, are used for the factor pair concerned. Purple-pigmented plants are divided into two classes, reddish purple with red glumes and blackish purple with black glumes, represented by the factor pair *Qq*. Phenotypes are illustrated in color (10, pl. XLII).

So far as known to the writers, shallu and Leoti sorgo are the only commercial varieties grown in the United States that have the brown plant color. Shallu is an introduction, and the parentage of Leoti is unknown. It might have been derived from a shallu hybrid. In segregating populations, reddish-purple or blackish-purple plants are easily distinguished from brown plants after they have reached a stage where foliage diseases or injuries cause the development of spots on the leaf sheaths and blades. The characters can be distinguished in seedlings a few days after mechanical injury or by examination of injured or decaying roots. Seemingly there is no distinguishable effect of the factor pair *Qq* on leaf sheaths of brown (*pp*) plants, but an effect on glume color is readily apparent. On brown plants *Q* produces sienna-colored glumes instead of the red found in purple plants, whereas the allelomorph, *q* gives mahogany-colored glumes instead of black. In populations heterozygous for both of these plant colors, *Pp* and *Qq*, a 9 : 3 : 3 : 1 segregation is obtained.

LINKAGE OF *D*, *Rs*, AND *P*

Linkage between the genes for juiciness (*Dd*) and seedling stem color (*Rrs*) was observed by the writers at Chillicothe in 1930, and this observation was reported by Martin (4) in a summary of sorghum linkages published in 1936. Genes for juiciness and plant color (*Pp*) were found to be linked in 1935, and this was mentioned briefly in the 1936 Texas station annual report (5). Rangaswami Ayyangar

et al. (8) in 1937 reported experiments showing linkage between juiciness and purple plant color, with 30 percent crossing over.

No triple recessive (*drsp*) plant was located until the late summer of 1936, when one was found in the progeny of a Leoti \times Ajax cross. Crosses with two triple-dominant varieties, Brown kaoliang, S. P. I.³ 38085, and Chusan Brown kaoliang, S. P. I. 23231, were made the same year, but only a few seeds were obtained. One F_1 plant of each cross grown in a greenhouse at Washington, D. C., during the winter of 1936-37, supplied small F_2 populations at Chillicothe in 1937. Additional F_2 and F_3 populations were grown in 1938. In the latter season classification of seedling stem color was somewhat questionable and, instead of labeling the seedlings, one head of each plant was bagged and after maturity seed was planted at the base of the plant to determine seedling stem color factors of the parent. The phenotypes for juiciness and plant color were separated readily.

The single-factor inheritance of each character in these populations is shown in table 1. The parental combinations and recombinations of the three characters are given in table 2. The indicated order of genes, with cross-over percentages, is *D* (10.9) *Rs* (16.4) *P*. The total cross-over percentage (27.3) agrees satisfactorily with the 30 percent reported by Rangaswami Ayyangar et al. (8).

TABLE 1.—Total classes of juiciness (*Dd*), seedling stem color (*Rrsr*), and plant color (*Pp*) in F_2 and F_3 segregating populations of (Leoti \times Ajax—*drsp* line) \times Brown kaoliang grown in 1937 and 1938, showing monogenic segregation for each of the characters

Factors	Total population	Classes		χ^2
		Dominant	Recessive	
	Number	Number	Number	Percent
Juiciness, <i>Dd</i>	2,091	1,576	515	24.63
Seedling stem color, <i>Rrsr</i>	2,091	1,588	503	24.06
Plant color, <i>Pp</i>	2,091	1,576	515	24.63

¹ $P > 0.05$ for segregation of each factor pair.

TABLE 2.—Parental combinations and recombinations of juiciness (*Dd*), seedling stem color (*Rrsr*), and plant color (*Pp*) in F_2 and F_3 populations coupling phase of (Leoti \times Ajax—*drsp* line) \times Brown kaoliang grown in 1937 and 1938

Combinations and recombinations	Factors	Population		Percent
		Number	Total	
Parental combinations.....	$\left\{ \begin{array}{l} DRsP \\ drsp \end{array} \right.$	$\left. \begin{array}{l} 1,303 \\ 259 \end{array} \right\}$	1,562	74.701
Recombinations between <i>Dd</i> and <i>Rrsr</i>	$\left\{ \begin{array}{l} dRsP \\ Drsp \end{array} \right.$	$\left. \begin{array}{l} 108 \\ 79 \end{array} \right\}$	187	8.943
Recombinations between <i>Rrsr</i> and <i>Pp</i>	$\left\{ \begin{array}{l} DRsp \\ drsP \end{array} \right.$	$\left. \begin{array}{l} 165 \\ 136 \end{array} \right\}$	301	14.395
Double recombinations.....	$\left\{ \begin{array}{l} DrsP \\ dRsP \end{array} \right.$	$\left. \begin{array}{l} 29 \\ 12 \end{array} \right\}$	41	1.961
Total.....			2,091	
Recombinations and double recombinations.....	$\left\{ \begin{array}{l} Dd-Rrsr \\ Rrsr-Pp \end{array} \right.$			$8.943+1.961=10.904$
Total.....	<i>Dd-Pp</i>			$14.395+1.961=16.356$
				27.260

³ S. P. I. denotes accession number of the Division of Plant Exploration and Introduction, formerly Office of Foreign Seed and Plant Introduction.

Unfortunately the seedling albino reported by Karper and Conner (3) as linked with seedling stem color, with 41.34 percent crossing over, could not be obtained for studies with this linkage group. The remnant seed was not viable, and probably the character is lost.

INDEPENDENCE OF THE *D Rs P* GROUP FROM CERTAIN OTHER GENES REPORTED

The segregation in F_2 of two or all three members of the linkage group with certain other characters is shown in table 3. The contrasting phenotypes are presence and absence of spreader (*Ss*), colored and white seed (*Rr*), awnless and awned lemmas (*Aa*), normal and antherless flowers (*Alal*), normal and male sterile flowers (*Msms*), and starchy and waxy endosperms (*Wwx*). The segregations indicate independent inheritance or cross-over values so high that linkage cannot be detected. The inheritance of each of these characters has been reported as being a simple 3 : 1 segregation. Independent segregation of members of this linkage group with members of the *Q B Gs* linkage group was shown in an earlier paper (13).

TABLE 3.—Independent segregation in the F_2 generation of miscellaneous characters with members of the linkage group *D* (juiciness), *Rs* (seedling stem color), and *P* (plant color); counts accumulated over a period of years and from various crosses

Independent characters	Factors (segregating pairs)	Observed F_2 ¹					χ^2
		Total	<i>AB</i>	<i>Ab</i>	<i>aB</i>	<i>ab</i>	
		<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	<i>Number</i>	
Spreader.....	<i>Ss-Dd</i>	2,042	1,237	366	333	106	20.409
	<i>Rrs-Ss</i>	218	132	39	35	12	.151
Pericarp color.....	<i>Rr-Dd</i>	3,744	2,130	711	690	213	.785
	<i>Rr-Rrs</i>	2,674	1,529	506	486	153	.177
Awns.....	<i>Dd-Aa</i>	7,165	3,968	1,379	1,332	486	.647
	<i>Rrs-Aa</i>	3,895	2,163	766	710	256	.064
Antherless.....	<i>Aa-Pp</i>	888	464	168	197	59	1.041
	<i>Dd-Alal</i>	320	189	55	62	14	.413
Male sterile.....	<i>Pp-Alal</i>	99	65	10	19	5	.407
	<i>Dd-Msms</i>	58	35	11	10	2	.572
Waxy.....	<i>Rrs-Msms</i>	109	57	19	25	8	
	<i>Pp-Msms</i>	377	234	57	74	12	1.598
	<i>Dd-Wwx</i>	1,822	1,120	286	329	87	.077
	<i>Rrs-Wwx</i>	2,202	1,306	331	455	110	.134
	<i>Pp-Wwx</i>	511	291	79	106	35	.910

¹ Collins' (1) formula was used to calculate expected classes.

² $n=2$, to obtain P value; $P>0.05$ in each case.

Collins' formula (1) was used to calculate expected classes in table 3. P values for deviations from calculated classes are in all cases greater than 0.05.

SUMMARY

This paper reports a linkage group in sorghum of three pairs of genes. The contrasting phenotypes are dry and juicy stalks, red and green seedling stem color, and purple and brown plant color. The factor pairs have been designated *Dd*, *Rrs*, and *Pp*, respectively, in other papers. Each of these factor pairs shows complete dominance in the F_1 and a 3 : 1 segregation in the F_2 . The indicated order of genes, with cross-over percentages, is *D* (10.9) *Rs* (16.4) *P*.

Although in some cases the numbers were small, independent inheritance or cross-over values so high that linkage could not be

demonstrated was found for two or three members of the linkage group with each of the following pairs: Presence and absence of spreader (*Ss*); colored and white seed (*Rr*); awnless and awned lemmas (*Aa*); normal and antherless flowers (*Alal*); normal and male sterile flowers (*Msms*); and starchy and waxy endosperms (*Wwx*).

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EFFECTS OF NITROGEN COMPOUNDS AND TRACE ELEMENTS ON GROWTH OF *ASPERGILLUS NIGER*¹

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INTRODUCTION

A further study of the essentiality of molybdenum (13)² and gallium (14) to *Aspergillus niger* Van Tiegh. and of the existence of a direct relation between molybdenum and nitrate utilization is reported in this paper.

It is generally accepted that ammonia is the form in which nitrogen serves for the elaboration of organic nitrogen by plants, other forms first undergoing transformation to ammonia. Nevertheless there exist in the literature statements to the contrary, the experimental foundations of which still lack other explanation. Kossowicz (6) concluded that when grown on *d*-mannitol and nitrite, *Aspergillus niger* and other fungi utilized nitrite directly without previous reduction to ammonia. The studies of Lemoigne and his coworkers (7, 8, 9), on the other hand, have led these investigators to believe that hydroxylamine is the form of inorganic nitrogen converted by the organism into organic nitrogen.

In the present investigation a study was made of growth with practically all the known forms of inorganic nitrogen and with certain simple forms of organic nitrogen. The oversight sometimes made by previous investigators of neglecting to test the nutrient solution, just prior to inoculation, for purely chemical transformations has led to the assumption that all the compounds present were due to biological action. Perhaps the most serious question raised involves the existence in plants of nitrite and hydroxylamine under normal conditions.

The existence of a nitrate-reducing enzyme in plants has been questioned by several investigators and the suggestion has been made that ascorbic acid in the plant serves this function (8). Quastel et al. (12) report that the ability of bacteria to reduce nitrate is destroyed by heat and acid, conditions not particularly conducive to the destruction of ascorbic acid. Though undeniable evidence of the existence of nitrate reductase is still to be obtained, there is no evidence that ascorbic acid participates in this reduction to any greater extent than other organic materials of the cell of similar capabilities. Mikhlin (10) states that reduction of nitrate with ascorbic acid does not proceed to ammonia and that *Chlorella* forms ammonia from nitrite though ascorbic acid appears to be absent. The fact that many organic substances, as well as light and heat, are known to decompose nitrates and nitrites makes tests with the isolated enzyme desirable.

¹ Received for publication August 9, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 747.

REVIEW OF LITERATURE

Since the publication of previous papers by the writer, DeRose et al. (2) and Hoagland and Arnon (5) have stated that they have found molybdenum essential for green plants. Mulder (11) has reported that he was able to verify the necessity of molybdenum for *Aspergillus niger* when nitrogen is supplied as nitrate. Arnon (1) found, moreover, that a mixture of elements containing molybdenum but not gallium was beneficial to the growth of barley. Also of interest is the conclusion of Dingle and Sheldon (3) that "molybdenum in minute traces is possibly a normal constituent of milk."

EXPERIMENTAL METHODS

The W strain of *Aspergillus niger* was grown in 50 cc. of a 5-percent sucrose solution in 200-cc. pyrex flasks for 4 days at 35° C. Information concerning the kind and quantities of mineral salt constituents added to the nutrient solutions is given with the experimental data. The compounds employed included sucrose containing not more than 0.0025 percent of ash, reagent mineral salts, and water redistilled in pyrex glass. Spectroscopically pure salts of the trace elements (Fe, Zn, Cu, Mn, Mo, and Ga) were used in one series of experiments. Sodium hyponitrite and sodium salt of nitrohydroxylaminic acid were prepared and furnished through the courtesy of Dr. Joseph Rosin, of Merck & Co. Other compounds of nitrogen tested are in commercial production. The cultures when harvested were filtered through fritted glass crucibles (IG₃), given a preliminary drying in a current of air at 45°, and dried overnight at 103°. Previous publications by the writer (13, 14) should be consulted for omitted details.

TRACE-ELEMENT EFFECTS WITH VARIOUS NITROGEN SOURCES

SPECIFIC EFFECT OF MOLYBDENUM

The specific effect of molybdenum in the reduction of nitrate nitrogen has been discussed previously (13). Exceptionally low yields were obtained when molybdenum was omitted in nutrient solutions containing nitrates of lithium, sodium, potassium, or magnesium. These results have been extended to include the nitrates of calcium, strontium, and barium (table 1).

The toxicity of strontium and barium to *Aspergillus* was either slight or nonexistent in these experiments. The former gave maximum yields, the latter yields that could be increased appreciably, as shown by untabulated data. Cystine hydrochloride was used as a source of sulfur in these experiments to avoid the precipitation of sulfate by strontium and barium.

The specific effect of molybdenum is not unique with nitrates, as formerly assumed, but is also given by nitrites and the sodium salt of nitrohydroxylaminic acid, as described later. Besides demonstrating the ineffectiveness of the cations Ca, Sr, and Ba in influencing results with molybdenum and nitrate, these data emphasize the biological specificity of molybdenum.

TABLE 1.—Effects of trace-element deficiencies on *Aspergillus niger* grown with various sources of nitrogen at 35° C. for 4 days 1.—Continued

Element omitted	Strontium nitrate ² Sample 3			Barium nitrate			Sodium nitrite			Hydroxylamine ³ hydrochloride			Sodium salt of nitrohydroxylaminic acid		
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁴	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁴	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁴	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest
Fe	Mili-grams 456.3	Percent 42.24	pH 3.28	8, bl	Mili-grams 253.4	Percent 30.62	pH 2.50	2, bl	Mili-grams 290.6	Percent 63.93	pH 3.15	8, bl	Mili-grams 150.7	Percent 32.17	pH 3.01
Zn	44.9	4.15	3.27	2, bl	129.9	17.05	3.06	10, bl	99.0	21.78	3.14	6, bl	238.2	51.09	3.28
Cu	1,106.9	102.46	2.84	4, t	508.1	66.66	3.23	0	477.3	103.02	2.84	6, bl	463.2	99.15	3.50
Mn	1,008.3	98.88	2.32	0	424.4	55.08	2.79	0	480.3	107.00	2.89	10, bl	506.5	108.37	2.90
Mo	1,078.4	99.82	2.94	8, bl	68.7	9.14	2.85	0	148.4	100.52	2.28	6, bl	150.9	32.29	2.73
None	1,080.3	100.00	3.02	8, bl	762.3	100.00	2.89	8, bl	494.5	100.00	2.33	6, bl	467.2	100.00	3.58
Maximum ⁵	1,185.3	47.41	3.46	---	945.8	37.83	2.97	---	495.0	19.80	3.75	---	514.9	20.60	2.19
C, U ⁶	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
pH ⁷	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

¹ Nitrogen was used at a concentration of 720 mg. per liter, except sodium nitrite at 2.0 gm., hydroxylamine hydrochloride at 0.5 gm., and sodium salt of nitrohydroxylaminic acid at 1.57 gm. per liter. Other constituents were sucrose at 50 gm. per liter; KH_2PO_4 and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ at 0.55 gm. and 0.25 gm. per liter respectively; and Fe, Zn, Cu, Mn, and Mo, at 0.20, 0.20, 0.05, 0.02, 0.02 mg. per liter respectively. Cysteine hydrochloride was substituted for sulfate ion in equivalent amount in the experiments with strontium and barium nitrates.

² Samples from different manufacturers.

³ Fungus harvested after 22 days.

⁴ Sporulation is indicated on a scale of 0 (sterile) to 10 (black with spores), and spore color by the initial letter or first 2 letters of the words white, yellow, tan, brown, and black.

⁵ Maximum individual yield.

⁶ Coefficient of utilization, or yield per 100 gm. of sucrose.

⁷ Initial acidity of the culture solution.

VARYING TRACE-ELEMENT CONTENT OF NITRATES

Table 1 shows also the wide variation in response to trace-element deficiencies with salts obtained from different manufacturers. The percentages of maximum yield obtained with calcium nitrate varied over 40 percent in minus-manganese cultures and nearly 87 percent in minus-molybdenum cultures. Data for still another sample of calcium nitrate will be found in the article (13) last mentioned. A similar marked variation in percentage of maximum yield in minus-molybdenum cultures was obtained with strontium nitrate.

RELATIVE VALUE OF NITROGEN SOURCES

Hydroxylamine proved a poor source of nitrogen. Sodium nitrite can also be so characterized, since chemical tests indicated that at the acidity necessary for growth decomposition occurred even prior to sterilization. A decided odor of nitric oxide could be detected, and tests revealed the presence of nitrate, hydroxylamine, ammonia, and residual nitrite. A somewhat similar situation exists in the utilization of potassium cyanate (see table 3), since at an acidity sufficient to permit growth complete destruction of cyanate with concomitant formation of ammonia had taken place. The effects of molybdenum deficiency with nitrite and with nitrohydroxylaminic acid salt indicate quite clearly that this element continues to function in the further reduction of these reduction products of nitrate.

YIELDS IN OPTIMUM SOLUTIONS CONTAINING GALLIUM

In the experiments of table 2 spectroscopically pure, instead of reagent, trace elements were used, and the data were extended to include the effects of a deficiency of gallium. Sucrose of 0.0014 percent ash extracted with 95-percent alcohol was employed. While these experiments were effective in demonstrating the need for gallium by *Aspergillus niger* under a variety of conditions in pyrex glassware and of the suitability of various procedures for such tests, the specific molybdenum-nitrate response was no longer apparent. It seems probable, therefore, that the solution of spectroscopically pure gallium used contained a minute trace of molybdenum. Though this interpretation may seem farfetched, the opposite is true. No molybdenum, for example, could be found in the ash of the mycelial felts nor in the reagents with a Bausch and Lomb large-size quartz spectrograph by Dr. B. C. Brunstetter, of the Bureau of Plant Industry. Similar tests on reagents by Dr. Joseph Rosin, of Merck & Co., were also negative, and were attributed by him to the lack of sensitivity of the Bausch and Lomb medium-size quartz spectrograph used. His estimate that double the sensitivity would be required to detect the presence of molybdenum in the reagents is in all probability too low. A small molybdenum deficiency is shown in each of the solutions, but one corresponding only to a maximum deficiency measured as loss in yield of 27 percent instead of 90 percent. The assumed trace of molybdenum in the gallium solution, of course, supplements that added with the other constituents of the solution.

TABLE 2.—Effects of trace-element deficiencies, particularly gallium, on *Aspergillus niger* grown with various nitrogen sources at 35° C. for 4 days ¹

Element omitted	NH ₄ NO ₃				NH ₄ Cl				NaNO ₃				Urea			
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ³	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ³	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ³	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ³
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe.....	220.3	25.29	2.00	6, bl	541.5	51.66	1.66	0, bl	298.3	31.17	3.25	8, bl	298.3	31.20	2.49	8, bl
Zn.....	35.8	4.11	2.76	6, bl	33.1	3.16	2.69	4, bl	623.9	97.50	3.23	2, bl	39.8	3.50	2.74	2, bl
Cu.....	567.2	55.12	1.85	6, bl	870.4	83.05	1.47	4, bl	823.9	97.50	2.68	6, br	39.8	3.50	2.22	6, br
Mn.....	484.5	55.03	1.79	2, bl	623.3	59.47	1.41	4, bl	824.4	85.84	2.40	8, bl	1,036.4	91.82	2.09	6, bl
Mo.....	674.6	77.45	1.88	4, bl	802.9	76.41	1.42	4, bl	694.7	72.60	3.09	8, bl	1,076.4	85.79	2.06	10, bl
Ga.....	534.0	68.20	1.88	4, bl	938.9	90.40	1.45	4, bl	743.0	77.65	3.15	8, bl	508.6	52.59	2.27	10, bl
None.....	871.0	100.00	1.90	4, bl	1,048.1	100.00	1.46	4, bl	956.9	100.00	2.49	10, bl	1,138.2	100.00	2.31	10, bl
Maximum ⁴	941.0	37.66	7.26	-----	1,091.3	43.65	7.22	-----	975.1	39.00	7.59	-----	1,169.9	46.68	7.64	-----
C, U, S.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Element omitted	NH ₄ NO ₃ (purified with CaO) ²				NH ₄ Cl (purified with CaO) ²				NaNO ₃ (purified with CaO) ²				Urea (purified with CaO) ²			
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ³	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ³	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ³	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ³
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe.....	18.3	1.78	2.63	1, bl	6.0	0.53	2.77	1, bl	14.4	1.75	2.71	1, bl	21.9	2.72	2.81	0, bl
Zn.....	22.8	2.22	2.80	1, bl	3.0	0.28	2.74	1, bl	21.5	2.62	3.26	1, bl	44.7	5.56	2.89	0, bl
Cu.....	864.4	84.25	2.60	4, br	883.0	84.59	1.46	3, bl	871.0	106.27	2.77	10, t	633.7	78.85	2.79	3, br
Mn.....	300.3	29.25	1.76	0	514.5	49.26	1.60	0	609.4	74.04	1.87	0	104.2	20.43	3.19	0
Mo.....	900.3	87.68	2.00	2, bl	701.1	75.73	1.47	2, bl	709.9	86.55	2.70	10, bl	649.6	80.82	2.93	2, bl
Ga.....	565.3	55.06	1.78	6, br	910.2	87.13	1.42	2, bl	462.6	56.44	3.39	8, bl	421.7	52.47	2.87	1, bl
None.....	1,027.1	100.00	1.99	4, bl	1,044.6	100.00	1.43	4, bl	819.7	100.00	2.63	10, bl	803.7	100.00	2.18	8, bl
Maximum ⁴	1,070.3	42.81	7.44	-----	1,121.0	44.84	7.31	-----	896.5	35.86	7.22	-----	989.2	39.57	7.27	-----
C, U, S.....	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

¹ See footnote 1, table 1.² Somewhat higher concentrations of constituents were used to avoid non-trace-element deficiencies.³ See footnote 4, table 1.⁴ See footnote 5, table 1.⁵ See footnote 6, table 1.⁶ See footnote 7, table 1.

NUTRITIVE VALUE OF SOME FORMS OF NITROGEN

The experiments shown in table 3, p. 738, are largely a repetition of those shown in table 1, except that gallium was added in each case and an experiment with potassium cyanate (KCNO) was included. The latter experiment has been discussed in connection with table 1. It will be noted that the results with molybdenum deficiency are quite poor when nitrogen is supplied as nitrohydroxylaminic acid, but that they are somewhat better with nitrogen as nitrite. These results are attributed to the cumulative effect of molybdenum impurity and not to any difference in behavior of these types of nitrogen. Of the intermediate products of nitrate reduction—nitrous acid (HNO_2), nitrohydroxylaminic acid ($\text{H}_2\text{N}_2\text{O}_3$), hyponitrous acid ($\text{H}_2\text{N}_2\text{O}_2$), and hydroxylamine (NH_2OH)—nitrohydroxylaminic acid and hyponitrous acid proved least toxic. The former ($\text{H}_2\text{N}_2\text{O}_3$) was the better source of nitrogen for growth of the fungus, the latter ($\text{H}_2\text{N}_2\text{O}_2$) could not be utilized. Nitrohydroxylaminic acid, like nitrous acid, was found to give a positive test with potassium iodide-starch, Griess reagent, and diphenylamine. The iodine-Griess reaction, or Blom test, for hydroxylamine is also given by nitrohydroxylaminic acid. Though the sodium salt of hyponitrous acid ($\text{Na}_2\text{N}_2\text{O}_2$) did not support growth, it permitted excellent germination to take place. The addition after several weeks of a favorable nitrogen source or the transfer of a bit of the submerged mycelium to Czapek agar resulted in rapid and normal growth.

Because of their marked toxicity, the quantity of sodium nitrite was limited to 2.0 gm. per liter and that of hydroxylamine hydrochloride to 0.5 gm. per liter. Sodium salt of nitrohydroxylaminic acid, and sodium hyponitrite were employed in amounts equivalent to 360 mg. of nitrogen per liter. Use of higher concentrations of these two salts was practicable, but the quantity of available material was small. All the other forms of nitrogen were used in quantities corresponding to 720 mg. of nitrogen per liter. The length of the growth period was 4 days with readily available sources of nitrogen but was extended sufficiently to obtain approximately maximum growth with other nitrogen sources.

GROWTH ON *d*-MANNITOL

Growth on *d*-mannitol (table 4) was uniformly less than with sucrose. Response of the organism, however, on this carbon source was quite similar to that on sucrose, both with trace elements and with different sources of nitrogen. The sample of mannitol employed was apparently not so free from trace-element impurities as the sucrose ordinarily employed, judging from the deficiency results obtained. Nevertheless, the same trace elements were required with both sources of carbon. A definite difference seemed to exist, however, in the production of organic acids inasmuch as final acidities at harvest were comparatively low with mannitol. The high acidities with ammonium chloride are laid to the effect of residual chloride ion.

TABLE 3.—*Effects of trace-element deficiencies and oxidative level of nitrogen supply on the growth of Aspergillus niger grown at 35° C. for 4 days*¹

Element omitted	Sodium nitrite				Sodium salt of nitrohydroxylaminic acid ²				Sodium salt of nitrohydroxylaminic acid ³			
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁵	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁵	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁵
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe.....	148.3	34.95	3.06	6, bl	261.5	90.79	3.09	8, bl	269.0	75.33	3.17	8, bl
Zn.....	20.2	4.75	3.28	2, bl	261.8	90.90	3.07	8, bl	384.5	107.67	3.14	4, bl
Cu.....	432.6	101.95	2.71	10, br	358.3	124.41	3.11	8, bl	365.2	102.27	3.25	8, bl
Mn.....	541.3	127.57	2.45	8, bl	3.7	1.28	5.09	8, bl	549.0	153.74	3.47	8, br
Mo.....	191.8	45.21	3.03	8, bl	268.0	93.06	2.77	0	279.0	78.13	3.35	8, bl
Ga.....	410.6	96.77	3.03	10, bl	360.6	125.21	3.32	8, bl	359.3	100.61	3.43	8, bl
None.....	424.3	100.00	2.97	10, bl	288.0	100.00	3.09	8, bl	357.1	100.00	3.26	8, bl
Maximum ⁶	541.3				360.6				549.0			
C. U. ⁷		21.65				14.42				21.96		
pH ⁸			4.15				8.32				5.76	

Element omitted	Sodium salt of nitrohydroxylaminic acid				Hydroxylamine hydrochloride ⁴				Potassium cyanate			
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁵	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁵	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ⁵
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe.....	168.9	60.93	2.98	4, bl	107.4	76.55	2.24	6, bl	474.3	42.90	2.23	8, bl
Zn.....	177.7	64.10	2.98	4, bl	68.2	48.61	2.34	6, bl	710.2	64.24	1.94	10, bl
Cu.....	331.3	119.52	2.92	6, bl	134.8	96.08	2.22	6, bl	1,117.3	101.06	1.87	2, w
Mn.....	358.8	129.43	2.77	8, bl	119.9	85.46	2.13	1, bl	669.7	60.58	1.86	0
Mo.....	305.9	110.36	2.89	8, bl	158.3	112.83	2.20	6, bl	1,142.2	103.31	1.86	10, bl
Ga.....	268.3	96.78	2.94	8, bl	138.8	98.93	2.24	6, bl	1,131.5	102.35	1.86	10, bl
None.....	277.2	100.00	2.96	8, bl	140.3	100.00	2.22	6, bl	1,105.6	100.00	1.86	10, bl
Maximum ⁶	358.8				158.3				1,146.8			
C. U. ⁷		14.35				6.33				45.87		
pH ⁸			4.24				2.97				7.26	

¹ See footnote 1, table 1.² Fungus harvested after 19 days.³ Fungus harvested after 20 days.⁴ Fungus harvested after 21 days.⁵ See footnote 4, table 1.⁶ See footnote 5, table 1.⁷ See footnote 6, table 1.⁸ See footnote 7, table 1.

Chemical tests for inorganic nitrogen compounds in solution revealed that mannitol differed from sucrose in other ways besides being a poorer medium for growth. These differences were most marked with nitrite. Only minute traces of ammonia could be detected in the culture solutions as a rule, and the Griess test for nitrite was usually negative until some time had elapsed, when a strong reaction might be obtained. Ammonium nitrate cultures seemed free of nitrogen except for faint traces of ammonia and nitrite which increased in intensity with duration. The absence of ammonia in the cultures could of course be due to the direct utilization of nitrite for formation of organic nitrogen as postulated by Kossowicz (6). In view of the marked toxicity of nitrite, a more logical interpretation is that with retardation of acid production the reduction to ammonia proceeds so slowly that little or none accumulates under ordinary conditions. The decided lag in tests for inorganic nitrogen may be indicative of combination with mannitol. Another possibility lies in the existence of still other reduction products as yet unknown. Nevertheless ammonia with mannitol, as with sucrose, is a far better source of nitrogen than is nitrite.

TABLE 4.—Effects of trace-element deficiencies and source of nitrogen on *Aspergillus niger* grown on *D-mannitol* at 35° C.¹

Growth on <i>D</i> -mannitol with—																				
Element omitted	NH ₄ Cl (8 days)				NaNO ₃ (7 days)				NaNO ₃ (19 days)				NaNO ₂ (21 days)				NH ₄ NO ₃ (7 days)			
	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. of sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ²
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe.....	418.1	79.88	1.63	2, bl	318.3	67.26	5.83	8, bl	41.7	249.70	6.53	2, bl	98.2	45.11	6.98	36	230.3	26.16	2.00	6, b
Zn.....	175.8	33.58	2.06	4, bl	90.4	19.10	2.47	2, bl	15.0	89.82	6.55	2, bl	128.9	59.08	5.15	36	185.2	21.03	2.21	6, b
Cu.....	453.1	86.56	1.48	2, bl	562.0	118.76	4.15	6, t	19.7	117.96	6.37	2, br	426.0	193.72	5.22	36	798.4	90.67	2.23	2, bl
Mn.....	608.7	116.30	1.41	2, bl	305.8	64.62	4.64	6, bl	26.5	158.68	6.82	2, br	598.3	272.07	4.17	36	490.1	53.60	2.01	2, bl
Mo.....	591.0	112.92	1.38	2, bl	444.1	93.85	4.91	10, bl	6.1	36.52	6.60	1, w	202.0	91.86	6.67	36	531.2	60.33	1.89	2, bl
Ca.....	464.8	88.80	1.42	2, bl	537.3	113.55	6.32	10, bl	9.1	54.49	7.08	2, br	315.5	143.47	7.31	36	735.7	83.56	2.70	4, bl
Ga.....	523.4	100.00	1.47	2, bl	473.2	100.00	5.32	8, bl	16.7	100.00	6.46	2, br	219.9	100.00	7.31	36	880.5	100.00	3.97	6, bl
None.....																	913.1			
Maximum ⁴	608.7				573.5				41.7				598.3					36.52		
C, U ³		24.35				22.94				1.67				23.93		4.03			3.92	
pH ⁵			4.03				2.24				7.59									

¹ See footnote 1, table 1.² See footnote 1, table 1. Mannitol at 50 gm. per liter was used instead of sucrose.³ See footnote 6, table 1.⁴ See footnote 4, table 1.⁵ See footnote 7, table 1.⁶ Spores yellow to black.

REDUCTION PRODUCTS OF NITRATE

COMPOUNDS OF NITROGEN WITH HYDROGEN OR OXYGEN

There are 19 known compounds of nitrogen containing only hydrogen or oxygen or both. They include 5 compounds generally considered to be reduction products of nitric acid, 8 oxides, hydrazine, hydrazoic acid, nitramide, pernitric acid, and hydronitrous acid. The 5 members of the nitrate reduction series are nitrous acid (HNO_2), nitrohydroxylaminic acid ($\text{H}_2\text{N}_2\text{O}_3$), hyponitrous acid ($\text{H}_2\text{N}_2\text{O}_2$), hydroxylamine (NH_2OH), and ammonia. Growth of *Aspergillus* was tested with all of the 19 compounds except 7 of the oxides (N_2O was included), nitramide, pernitric acid, and hydronitrous acid. Since the oxides of nitrogen other than nitrous oxide decompose in aqueous solution with formation of members of the nitrate reduction series, their omission was unimportant. Nitramide could not be obtained, since it undergoes violent decomposition at 72°C .; pernitric acid as an oxidation product of nitric acid was unnecessary; and hydronitrous acid does not exist in the presence of water. The nitrogen compounds included are considered, therefore, to comprise a complete list of the known possibilities. A number of organic nitrogen compounds were also included in these tests.

ASSIMILABILITY OF NITRATE-AMMONIA SERIES

The members of the nitrate-ammonia series differ widely in their acceptability to *Aspergillus* as a source of nitrogen. Nitrates, ammonium salts, and sodium salt of nitrohydroxylaminic acid provide excellent nontoxic sources of nitrogen available over a wide range in acidity, whereas nitrite and hydroxylamine are quite toxic and practically useless for growth. The reason for characterizing nitrite as useless is that at an acidity permitting growth the nitrite is decomposed with the formation of nitrate and ammonia. Growth does not take place on nitrite if the solution is neutral or only slightly acid. Hydroxylamine is also quite toxic and could not be used in concentrations over 0.5 gm. per liter, and requires markedly acid solutions to permit slight growth. Spore germination in cultures with sodium hyponitrite is excellent, but practically no growth takes place because, although the hyponitrite is nontoxic, its nitrogen is unavailable.

NITROGEN COMPOUNDS IN NUTRIENT SOLUTIONS

The precaution was taken of testing the various nutrient solutions for the presence of the different forms of inorganic nitrogen. The chemical tests that were used are shown in table 5, and were verified with the nitrogen compounds as soon as they were received. It is important to note, particularly, that nitrohydroxylaminic acid gives every positive test given by nitrous acid, besides giving a positive iodine-Griess, or Blom test. Boiling the solution with sulfanilic acid to destroy nitrite evidently results in the production of some hydroxylamine from nitrohydroxylaminic acid, since a positive Griess test is given by its subsequent oxidation to nitrite with iodine.

TABLE 5.—Qualitative tests for some forms of inorganic nitrogen in dilute solution ¹

Nitrogen compound ²	Reaction with—				
	Starch-potassium iodide	Griess nitrite test	Diphenylamine	Iodine-Griess test ³	Nessler reagent
Nitrate.....	—	—	+	—	—
Nitrite.....	+	+	+	—	—
Salt of nitrohydroxylaminic acid.....	+	+	+	+	—
Hyponitrite.....	⁴ ±	⁵ —	—	+	—
Hydroxylamine.....	—	—	—	+	+
Ammonia.....	—	—	—	—	+

¹ The procedures used in making these tests are described in Fred and Waksman's manual (4). A positive reaction is indicated by +, a negative by —. The salts were tested immediately upon receipt.

² The gases NO₂ and NO are reported to give positive starch-iodide and Griess tests.

³ Iodine is used to oxidize hydroxylamine, etc., to nitrite before testing.

⁴ Usually stated to be negative; test faint but definite.

⁵ Usually stated to be positive.

A positive Griess test indicates the presence of either nitrous acid or nitrohydroxylaminic acid; a positive diphenylamine-sulfuric acid test may be due to the presence of nitric, nitrous, or nitrohydroxylaminic acids; and a positive Blom test indicates the presence of nitrohydroxylaminic acid, hyponitrous acid, or hydroxylamine. The positive ammonium test with hydroxylamine is probably due to the presence of decomposition products, since hydroxylamine is quite unstable.

The results obtained with these chemical tests of nutrient solutions just prior to inoculation are given in table 6. The nonspecificity of these tests should be borne in mind. It is clear, however, that some decomposition of these nitrogen compounds had taken place in culture solution before inoculation and that the products differed with the acidity. A possible connection may exist between the faculty of preinoculation formation of ammonia and availability of the compound for growth, since hyponitrous acid shows no trace of ammonia formation nor does nitrous acid in neutral solution. The nitrogen compounds identified after growth has taken place may therefore not always be due to biological action, as some investigators have assumed.

TABLE 6.—Preinoculation nitrogen decomposition products in nutrient solutions containing sucrose ¹

Nitrogen compound added	Preinoculation decomposition products in acid solution	Preinoculation decomposition products in alkaline or neutral solution	Supplementary nitrogen compounds aiding growth
Nitrate.....		HNO ₂ , (NH ₃ ?)	
Nitrite.....	HNO ₃ , NH ₃	HNO ₃ , NH ₂ OH	None.
Nitrohydroxylaminic acid salt.....	HNO ₂ , NH ₃	HNO ₃ , HNO ₂ , NH ₂ OH	NH ₂ OH (slight).
Hyponitrite ²	HNO ₂ , NH ₂ OH	(HNO ₂ ?), (NH ₂ OH?)	NH ₃ , HNO ₃ , urea, H ₂ N ₂ O ₃ .
Hydroxylamine.....	NH ₃	NH ₃	NH ₃ .
Ammonia.....			

¹ The tabulated results will probably differ under different conditions.

² Addition of $\frac{N}{10}$ NaOH previous to Nessler test resulted in a strong ammonia reaction.

The last column of table 6 indicates the compounds of nitrogen that serve to increase yield when added to approximately neutral nutrient solutions provided with nitrogen of the form stated in the first column.

An attempt has been made to distinguish between the supplementary nitrogen compounds that bring about a rapid increase in the rate of growth and those causing a final increase without markedly influencing the rate. It is those falling within the former group only that are listed, since inclusion of the latter group would, except with nitrite, necessitate the addition of practically all nitrogen compounds tested. The rate of growth is maximum with ammonium and nitrate nitrogen and these were not tested. Toxicity is probably the cause of the negative results with nitrite, just as only nitrogen deficiency with hyponitrite is the reason for positive results with most of the compounds tested. The beneficial action of hydroxylamine hydrochloride on growth with sodium salt of nitrohydroxylaminic acid is attributed to the marked acidity of the hydrochloride. Some chemical interaction may be responsible, however. Hydroxylamine, therefore, was the only nitrogen source showing a definite response when supplemented with another source of nitrogen (NH_3), which seems to indicate that reduction and not oxidation is most beneficial in improving the assimilability of hydroxylamine by *Aspergillus*.

The state of reduction of inorganic nitrogen would seem to be without intrinsic effect upon the formation of mass per unit of nitrogen supplied, a result that also seems to indicate that these various compounds are transformed into a single compound for elaboration into organic nitrogen. If the maximum yield per milligram of nitrogen supplied is computed it is found that nitrate, ammonium, and hydroxylamine form approximately 33 mg. of mass. With nitrite the fungus formed 30 mg. of mass per milligram of nitrogen, and with nitrohydroxylaminic acid approximately 31 mg. of mass. The slightly lower yields may be due to experimental error or to chemical side reactions.

Hydrazine sulfate ($\text{N}_2\text{H}_4\cdot\text{H}_2\text{SO}_4$) and sodium azide (N_3Na) were unsuitable for supplying nitrogen in culture, except as supplementary sources of nitrogen. Nitrous oxide also failed to support growth. The following organic substances were tested and found to give little growth: *n*-heptaldoxime, acetone oxime, dimethylglyoxime, urethane, methylurea, phenylurea, diazoaminobenzene, azobenzene, and sulfamic acid. No germination occurred with *s*-diphenylurea, azoxybenzene, hydrazobenzene, and methylhydrazine sulfate. Therefore, the attempts to supply the fungus with sufficient hydroxylamine at low concentrations for maximum growth by using difficultly soluble oximes that might furnish this compound through slow hydrolysis proved unsuccessful. Moreover, the tests would seem to indicate that substitution of organic radicals for hydrogen in urea and in the members of the nitrate reduction series practically prevents their use as a source of nitrogen. Neither did substitution of radicals in hydrazine and hyponitrous acid lead to any improvement in nutritiveness.

POST-GROWTH METABOLIC PRODUCTS IN CULTURE

CONSTITUENTS FOUND

The tests shown in table 5 were also employed to identify inorganic nitrogen compounds in solution at the time of harvest. In addition, urea was sought for with xanthidrol and the presence of soluble amino nitrogen compounds with phosphotungstic acid containing 5 percent

of sulfuric acid. Also included were tests for starch in both mycelial felt and culture solution. It is not known what substances are responsible for the results of the ferric chloride test. All reactions were carried out on porcelain test plates with about 0.5 ml. of solution, and were essentially qualitative. The quantities of precipitate and degrees of coloration afforded a rough measure of quantities concerned, however. The results obtained will be found in table 7.

In control cultures with any source of nitrogen suitable for growth there were usually found various derived nitrogen compounds, usually, with one exception, reduction products. However, hydroxylamine was formed from ammonium salts with sucrose but not with *d*-mannitol. The formation of urea and of soluble amino acids or their condensation products was also general. A marked difference in starch (and amino acid) formation was found between solutions with ammonium and nitrate nitrogen in that starch was formed profusely with ammonium, whereas little or no starch was formed with nitrate. The starch responses with urea and potassium cyanate resembled those with ammonium, whereas with nitrate, nitrohydroxylaminic acid, and hydroxylamine these responses resembled those with nitrate. Acidity was an important factor in starch formation also.

EFFECTS OF TRACE-ELEMENT DEFICIENCIES

The effects of a trace-element deficiency were not always the same, neither was the extent of the deficiency on yield. Generally, the greater the deficiency the smaller were the quantities of products formed and the greater were the concentrations of residual ions initially added. Low yields because of iron deficiency were frequently accompanied by an increased hydroxylamine production, whereas low zinc prevented its formation. A phenomenon that was noticed repeatedly, but not invariably, was associated with molybdenum deficiency in ammonium nitrate solutions containing gallium. A low yield (73 percent or less) was accompanied in these cases by high acidity and profuse starch formation, the residual ammonia being relatively low and the residual nitrate high. The data in table 7 do not show this clearly, but the dibasic optimum solution purified with di- or tri-calcium phosphate can generally be depended upon to give this response. The increased acidity in minus-molybdenum cultures may possibly contribute to the increase in starch formation, but does not seem to effect reduction of nitrate to any extent. Manganese deficiency leads to similar responses that differ only in the formation of an immediate brilliant-blue test for starch in the felts, whereas low molybdenum gives a brown iodine test with the mycelial felt that changes to a deep blue eventually. Though these may be wholly nonspecific deficiency responses, the very fact that omission of an element causes such a response would afford additional evidence of the essentiality of that element to growth.

TABLE 7.—Results of tests for various metabolic products of *Aspergillus niger* in solution at time of harvest ¹

Constituent found in cultures at time of harvest	Element omitted from solution with—																							
	Ammonium nitrate						Ammonium chloride						Sodium nitrate						Urea					
	None						None						None						None					
	Fe	Zn	Cu	Mn	Mo	Ga	Fe	Zn	Cu	Mn	Mo	Ga	Fe	Zn	Cu	Mn	Mo	Ga	Fe	Zn	Cu	Mn	Mo	Ga
Ammonia	3	3	0	4	2	3	3	3	1	3	2	1	1	1	1	1	1	1	1	0	1	0	0	0
Hydroxylamine	2	2	0	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Nitrite	2	2	0	0	0	0	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Nitrate	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Urea	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Starch in mycelial felt	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Starch in culture solution	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Ferric chloride test	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Phosphotungstic acid precipitate	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Constituent found in cultures at time of harvest	Element omitted from solution with—												Element omitted from solution with—											
	Hydroxylamine						Potassium cyanate						d-Mannitol and sodium nitrate						d-Mannitol and sodium nitrite					
	None						None						None						None					
	Fe	Zn	Cu	Mn	Mo	Ga	Fe	Zn	Cu	Mn	Mo	Ga	Fe	Zn	Cu	Mn	Mo	Ga	Fe	Zn	Cu	Mn	Mo	Ga
Ammonia	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Hydroxylamine	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Nitrite	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Nitrate	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Urea	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Starch in mycelial felt	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Starch in culture solution	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Ferric chloride test	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Phosphotungstic acid precipitate	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

¹ Quantity of precipitate and intensity of coloration are indicated on a scale of 0 (none) to 5 (very strong); E refers to presence of erythroductrin.

DISCUSSION

The necessity of molybdenum for the growth of *Aspergillus* is made still more certain by the markedly poor growth of the fungus when this element has not been added to nutrient solutions containing sodium nitrite and sodium salt of nitrohydroxylaminic acid. It may be assumed, therefore, that molybdenum is necessary for the reduction of these compounds as well as of nitrate. The absence of positive results with sodium hyponitrite and with hydroxylamine requires further elucidation, however. The effect of molybdenum deficiency with ammonium nitrate is assumed to depend on the "locking" of nitrate, so that the organism is actually growing in effect with ammonium nitrogen. Further chemical work of a quantitative nature will be necessary for a complete understanding of the processes involved. The very existence of the molybdenum-nitrate effect would indicate the essentiality of this element for growth. The diminished effect of withholding molybdenum when gallium is added is not attributed to gallium but to the presence of impurities. Other experiments not reported here have resulted in diminutions in yield of more than 60 percent.

The data on gallium deficiency contain nothing intrinsically new, since they are but an extension and amplification of the data formerly reported (11). The nonaddition of gallium to the nutrient solution resulted in a lessened growth in certain instances, but the effects were slight except under the best of conditions, namely, the experiments in table 2, in which sucrose extracted with 95-percent alcohol was used.

The results of the experiments with the reduction products of nitric acid are taken to indicate that ammonium is the only source of inorganic nitrogen of direct service to the fungus for the elaboration of organic nitrogen. Other sources of nitrogen are converted into ammonia before use, and their nutritiveness would seem proportional to the rate and extent of their conversion.

Tests with mixed nitrogen sources were made on the assumption that rate of formation of reduction or oxidation products in the nitrate-ammonium series depends on initial concentration of the compound undergoing reaction and the rate of removal of the final product (law of mass action). Within the limits fixed by the requirements of the organism only the addition of ammonia to hydroxylamine proved effective. Since hydroxylamine was employed at the maximum concentration its toxicity permitted, the increased yield cannot be attributed to an increase in hydroxylamine concentration through oxidation of ammonia. It is considered, on the contrary, as a demonstration that hydroxylamine is employed by the fungus only after reduction to ammonia.

The association between assimilability and decomposition with acid to form ammonium with the reduction products of nitrate would seem to have a similar interpretation. The poor growth with nitrite at low acidities is no exception if the toxicity of nitrite be considered. At high acidity, moreover, nitrite is also decomposed with the formation of ammonium. The only evidence obtained for the formation of ammonium from hyponitrite was under alkaline conditions entirely unsuitable for growth of the fungus. However, these compounds are highly unstable, decomposition sometimes proceeding at an explosive rate, and the products formed depend largely on the conditions of the experiment.

Acid decomposition of nitrous and nitrohydroxylaminic acids probably is not the primary reaction employed for the production of ammonia by the organism, since growth in acid solution is quite meager in the absence of molybdenum. Nevertheless proof that molybdenum is not concerned in the purely chemical production of ammonia by acid in the nutrient solution would strengthen the interpretation that these reduction processes are really enzymatic.

The considerable evidence that has accumulated for the existence of nitrite and hydroxylamine in the tissues of green plants and micro-organisms would seem to require further verification in the light of these results. The data regarding these compounds are more logically explained on the assumption of the presence of nitrohydroxylaminic acid. This compound is also a reduction product of nitrate and is, above all, of little toxicity. The presence of hydroxylamine among its decomposition products formed by acidity would readily account for the identification of this relatively toxic compound in the tissues of organisms.

The experimental results of Kossovitz (6) with nitrite nitrogen and *d*-mannitol were duplicated. They cannot be accepted as evidence for the direct utilization of nitrite nitrogen for several reasons, however. Nitrites are quite as toxic with mannitol as with sucrose. A slower rate of reduction to ammonia with mannitol could also serve to explain these experimental results, especially if combined with the failure of growth to cause a normal increase in acidity of the cultures. The consistent presence of traces of ammonia in the cultures renders that interpretation not improbable. There is, in addition, the possibility that chemical combination occurs between mannitol and nitrite at least, since the Griess test is long delayed. Similar lags in response were obtained with both the ammonia and nitrite tests when ammonium nitrate was the source of nitrogen.

The main objections to be met before the view can be accepted that hydroxylamine is the primary source of inorganic nitrogen would seem to be those based on its toxicity and slow assimilation. Its formation from inorganic nitrogen of higher oxidation level is to be more or less expected, but this in itself is no evidence of its utilization in this form. Its presence in solution in certain instances may be and probably is in the role of a waste product usually in association with high ammonium concentration. The formation of hydroxylamine from ammonium chloride must be interpreted upon some such basis. Oxidation of ammonium to hydroxylamine in the culture solution is questionable, since its reduction potential is sufficient for formation of ammonium. Molybdate is reduced to "molybdenum blue" and methylene blue becomes green.

The attractiveness of the hydroxylamine hypothesis consists in the ease with which α -ketonic acids may be converted to amino acids through oxime formation with hydroxylamine, and their subsequent reduction to amino acid and regenerated hydroxylamine. Amination of α -ketonic acids is also readily accomplished, however, by Knoop's method of catalytic reduction in the presence of ammonia. Additional evidence would be desirable, however, on the extent to which degradation of hexoses by organisms results in the formation of α -ketonic acids. Mammalian forms have been claimed to possess to a limited degree the ability to form amino acid from ammonium salts.

Repetition of some phases of these studies from the chemical viewpoint would seem desirable. Development of specific qualitative and quantitative methods would be of paramount importance, however. *Aspergillus*, in itself and because of its close correspondence to green plants as regards nitrogen utilization, furnishes excellent material for such investigations.

SUMMARY

Growth of *Aspergillus niger* in culture solution has led to additional proof of the essentiality of iron, zinc, copper, manganese, and particularly molybdenum and gallium. Molybdenum seems to be of special importance in the reduction not only of nitrates but of nitrites and nitrohydroxylaminic acid salt as well.

Data are presented to indicate the wide variation in trace-element content of different samples of reagent nitrates. Nitrates, ammonium salts, and nitrohydroxylaminic acid salt were found to be the best sources of inorganic nitrogen for growth. Nitrite, hyponitrite, hydrazine, azide, and nitrous oxide were useless for growth. Hydroxylamine was a poor source of nitrogen. The positive tests for nitrite in organisms are attributed to nitrohydroxylaminic acid, whose presence may also account for the hydroxylamine reported to be present.

Analogies were found between the ability of inorganic nitrogen compounds to form ammonia chemically, under the influence of acidity, and their assimilability. Substitutions of organic radicals in such compounds in no case improved assimilability. Ammonia is concluded to be the primary source of nitrogen for conversion to organic nitrogen.

Specific starch reactions associated with manganese and molybdenum deficiency are described as a phenomenon repeatedly noticed in the formation of post-growth metabolic products in culture.

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RELATION OF CARBON NUTRITION TO TRACE-ELEMENT AND ACCESSORY REQUIREMENTS OF *ASPERGILLUS NIGER*¹

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INTRODUCTION

Previous investigations by the writer (18)² on trace-element and accessory-factor needs of *Aspergillus niger* Van Tiegh. have been limited to nutrient solutions containing sucrose. Studies with other sources of carbon were considered desirable, therefore, for several reasons. The nature of impurities in different carbon compounds might vary sufficiently to give improved deficiency tests with some of the essential trace elements. On the other hand, the possibility existed that the trace-element requirements of the organism might vary quantitatively with modification in nutrition, as in the case of molybdenum and nitrate (16). Lastly, there was a further possibility that glycerol might prove an effective substitute for sucrose in the metabolism of the fungus and that it might be feasible to obtain it in a higher state of purity than sucrose by repeated distillation in the laboratory.

The present paper reports results obtained with glycerol and with various other carbon compounds.

REVIEW OF LITERATURE

A recent review of the literature has been published elsewhere by the writer (18), and only investigations of direct importance to these studies need be mentioned here. The review by Bonner (1) of recent progress in studies with accessory growth substances should also be consulted.

A tentative classification of carbon compounds on the basis of nutritive properties has been proposed in a preliminary report (20) of some of the results on organic nutrition presented here. The groups suggested were calogens (energy) and formogens (molecular configuration). The latter included protosins (structural), biosins (chemical), and hormones (regulatory). Carbon compounds were considered to possess these properties in varying degree and to be capable of altering and particularly of supplementing their mutual deficiencies. The results since obtained are also readily interpreted on the basis of this grouping as phenomena of malnutrition and are practically identical in import with the experimental data on accessory growth factors.

In the absence of definite standards for comparison, the relative nutritiveness of carbon compounds can only be stated in general terms. Sucrose, *d*-glucose, *d*-fructose, and *d*-mannose are usually found to be excellent sources of carbon; glycerol and *d*-mannitol are rated as fair; and *d*-galactose and *d*-lactose are generally found to be poor. Data on *l*-sorbose are lacking. Lactose on hydrolysis forms

¹ Received for publication September 8, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 762.

an equimolecular mixture of glucose and galactose, but Pottevin (9) found *Aspergillus niger* unable to utilize it for growth, though it sufficed for respiration with the mature felt. Failure to grow on lactose was attributed to nonformation of lactase in the immature felts. Its presence in the mature felts was ascertained by Pottevin.

Previous studies by the writer (15, 20) with the trace elements would indicate that the strain of *Aspergillus niger* employed requires no accessory growth factors and readily attains maximum normal growth with sucrose and inorganic nitrogen. Nielsen and Hartelius (6), and Nielsen and Sing-Fang (8) report the existence of another strain of this fungus requiring pyruvic and glycolic acids, however. Similar claims advanced by other investigators also would indicate the existence of variations in accessory requirements in the *Aspergillus niger* group similar to those existing among the yeasts. Nevertheless, the possibility that experimental accessory deficiency may in some cases be due to faulty carbon and nitrogen nutrition (20) and is not inherent in the organism has by no means been disproved.

All degrees of specificity in amino acid requirements for growth have been found to exist among the fungi. Papers by Ezekiel et al. (2), Nielsen and Hartelius (7), Leonian and Lilly (5), and others deal with this question. It is considered by Nielsen and Hartelius that β -alanine, *d*-lysine, *d*-arginine, and *d*-glutamic acid function with yeasts as accessory substances and not as sources of nitrogen. This would make a total of nine accessories required for growth of yeast, the others being *i*-inositol, aspartic acid, thiamin, pantothenic acid, and biotin (4). The writer (20) has pointed out the beneficial action of sodium iron chlorophyllin in conjunction with *i*-inositol on the growth of yeast; and with amino acids on the growth of *Aspergillus niger* with *d*-mannitol, *d*-lactose, and glycerol.

The mutual interdependence in composition of carbon and nitrogen compounds with respect to nutritiveness is not confined in all probability to determination of the supplementary amino acids and accessories required for growth by plants. Stirn and Arnold (21) were able to cure functional disturbances in rats due to thiamin deficiency by partial substitution of triacetin or tricaproin for carbohydrate. Of equal significance, perhaps, is the decrease in utilization of ingested lactose by the rat upon removal of butterfat from milk, reported by Schantz et al. (13). Whether other fungi found to require an external supply of thiamin, pyrimidine, or thiazole with a glucose-asparagine solution, as in the case of *Phycomyces* (10, 11, 12), will react similarly under all conditions remains to be determined. Thiamin deficiency in fungi may in some instances be associated with a high carbohydrate diet because of the presumable need for a greater quantity of thiamin than can be supplied at the rate at which the organism is capable of synthesizing it.

METHODS

The "W" strain of *Aspergillus niger* was grown on 50 cc. of a 5-percent sucrose solution in 200-cc. pyrex Erlenmeyer flasks for 4 days at 35° C. Ammonium nitrate, monopotassium phosphate, and magnesium sulfate (7H₂O) were also added to the nutrient solution in amounts of 2.06, 0.55, and 0.25 gm. per liter respectively. All chemicals were of reagent grade or the purest obtainable. The water

was redistilled in a pyrex glass still. Sterilization of media was effected by heating for 20 minutes in the steamer, except for cultures containing yeast extract. The latter were treated in the autoclave for 20 minutes at 15 pounds' pressure. The flasks were inoculated with a suspension of spores. The cultures were filtered with fritted glass crucibles of No. 3 porosity, and were dried overnight at 103° before weighing.

The organic substances tested for supplementary growth action included those listed in a previous paper (17) together with pimelic acid, nicotinic acid, androsterone, *d*-tocopherol, nutrose, yeast extract, malt extract, Witte peptone, Bacto-peptone, 3,5-*l*-diiodotyrosine, *dl*-threonine, and citric, glyceric, gluconic, glyoxylic, kojic, lactic, mucic, oxalic, and tartaric acids. Though 82 compounds were used, many known to be important in metabolism were unavailable for test. What other substances may be important in metabolism is of course as yet unknown. Another handicap consisted in the inability to obtain and test the decomposition products of the substances found to have a supplementary growth effect.

The method followed in the study of these organic substances was to determine which substance was the most effective and then test the effectiveness of the other substances when used in conjunction with it. This procedure was continued until growth was approximately maximum. Concentration of the individual supplementary substances was arbitrarily limited to 20 mg. per liter, i.e., 20 parts per million. This scheme was followed in its entirety, however, only with glycerol, and included thorough tests with a total of 77 chemical elements. In other cases the tests with the complete list of chemical elements were omitted, and, where an organic compound had been found capable of bringing about satisfactory growth, tests with the other organic substances were omitted as well.

Reference is made to purification experiments with glycerol. These were performed in a manner similar to that used with sucrose (14). A gram of calcium carbonate was added to the nutrient solution containing a slight excess of constituents, which was then heated and filtered while hot through a fritted glass filter of No. 4 porosity. This method was not employed with the other sources of carbon in the present series of experiments.

GROWTH WITH GLYCEROL

The data in table 1 are typical of the responses of *Aspergillus niger* when grown on glycerol. It will be noted that the yields in the first three experiments without scandium are 107.4, 57.5, and 21.7 mg. per culture, respectively, when the nutrient solution contained reagent glycerol, redistilled glycerol, or redistilled glycerol in a purified solution. Appreciable increases in yield were brought about through the addition of traces of scandium to the nutrient solution. These increases in growth could not be obtained with any of the other 76 chemical elements at the concentration levels tested. Moreover it would appear that the organism requires iron, zinc, copper, manganese, molybdenum, and gallium with glycerol as well as with sucrose. The poor results with trace-element deficiencies are considered to be due largely to the poor yields obtained relative to the level of trace-element impurity.

TABLE 1.—Effects of a deficiency of trace elements on the growth of *Aspergillus niger* for 4 days at 35° C. with glycerol and with glycerol to which traces of organic nitrogen compounds had been added¹

Element omitted	Glycerol											
	Reagent				Distilled				Distilled and purified with CaCO ₃			
	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²
	Milli-grams	Per-cent	pH		Milli-grams	Per-cent	pH		Milli-grams	Per-cent	pH	
Fe.....	102.7	38.12	2.03	2, bl	36.3	66.48	-----	2, bl	4.3	7.43	-----	2, bl
Zn.....	281.4	85.89	1.88	4, bl	17.3	31.68	-----	2, bl	7.7	13.30	-----	2, bl
Cu.....	305.3	113.33	1.72	2, bl	40.3	73.81	-----	2, bl	53.0	91.54	-----	2, bl
Mn.....	303.4	112.62	1.84	0	49.6	90.84	-----	2, bl	196.4	339.20	-----	2, bl
Mo.....	243.7	90.46	1.77	4, bl	53.5	97.88	-----	2, bl	55.1	95.16	-----	2, bl
Ga.....	241.2	89.53	1.87	2, bl	101.5	185.90	-----	2, bl	25.9	44.73	-----	2, bl
Se.....	107.4	39.87	2.01	2, bl	57.5	105.31	-----	2, bl	21.7	37.48	-----	2, bl
None.....	269.4	100.00	1.82	4, bl	54.6	100.00	-----	2, bl	57.9	100.00	-----	2, bl
Max. ³	387.6	-----	-----	-----	104.4	-----	-----	-----	196.4	-----	-----	-----
C. U. ⁴	-----	15.50	-----	-----	-----	4.18	-----	-----	-----	7.86	-----	-----
pH ⁵	-----	-----	5.30	-----	-----	-----	4.75	-----	-----	-----	6.33	-----

Element omitted	Glycerol with indicated nitrogen compound (20 mg. per liter)											
	Witte peptone				Bacto peptone				Sodium iron chlorophyllin			
	Yield	Proportion	Acidity	Sporulation	Yield	Proportion	Acidity	Sporulation	Yield	Proportion	Acidity	Sporulation
Fe.....	98.6	22.09	2.28	1, bl	72.0	21.44	2.33	2, bl	553.1	77.10	1.99	8, bl
Zn.....	455.3	102.02	1.89	1, bl	359.7	107.12	1.81	2, bl	301.5	42.03	1.91	10, bl
Cu.....	423.3	94.85	2.19	1, bl	448.5	133.57	2.28	2, bl	410.9	57.28	1.87	6, bl
Mn.....	903.1	202.35	3.79	1, bl	761.3	226.71	3.17	2, bl	422.6	58.91	1.77	8, bl
Mo.....	350.9	78.63	1.87	1, bl	280.1	83.41	1.87	2, bl	590.0	82.24	1.97	6, bl
Ga.....	446.1	99.95	2.18	1, bl	387.8	115.49	2.02	2, bl	684.1	95.36	2.29	6, bl
Se.....	455.2	102.00	2.21	1, bl	334.4	99.58	1.96	2, bl	691.0	96.31	2.31	6, bl
None.....	446.3	100.00	2.28	1, bl	335.8	100.00	1.99	2, bl	717.4	100.00	2.36	6, bl
Max. ³	903.1	-----	-----	-----	761.3	-----	-----	-----	744.3	-----	-----	-----
C. U. ⁴	-----	36.12	-----	-----	-----	30.45	-----	-----	-----	29.77	-----	-----
pH ⁵	-----	-----	4.67	-----	-----	-----	4.76	-----	-----	-----	5.01	-----

Element omitted	Glycerol with indicated nitrogen compound (20 mg. per liter)—Continued											
	L-Proline				d-Lysine				Hemoglobin			
	Yield	Proportion	Acidity	Sporulation	Yield	Proportion	Acidity	Sporulation	Yield	Proportion	Acidity	Sporulation
Fe.....	86.5	17.83	2.39	8, bl	78.6	18.37	2.41	8, bl	227.2	41.56	1.93	8, bl
Zn.....	174.4	35.94	2.05	10, bl	118.6	27.72	2.22	8, bl	289.9	53.04	1.89	8, bl
Cu.....	501.7	103.40	2.02	6, bl	217.2	50.77	1.90	6, bl	543.4	99.41	2.09	6, bl
Mn.....	253.8	52.30	1.86	6, bl	173.4	40.53	1.99	6, bl	451.8	82.65	1.87	6, bl
Mo.....	416.4	85.82	1.83	6, bl	356.5	83.33	1.80	6, bl	522.2	95.53	1.97	6, bl
Ga.....	617.3	127.23	2.26	6, bl	456.4	106.69	1.91	6, bl	596.7	109.16	2.17	7, bl
Se.....	552.4	113.85	2.16	6, bl	456.4	106.69	1.87	6, bl	582.3	106.53	2.09	7, bl
None.....	485.2	100.00	2.07	6, bl	427.8	100.00	1.92	6, bl	546.6	100.00	2.10	6, bl
Max. ³	617.3	-----	-----	-----	456.4	-----	-----	-----	619.3	-----	-----	-----
C. U. ⁴	-----	24.69	-----	-----	-----	18.26	-----	-----	-----	24.77	-----	-----
pH ⁵	-----	-----	4.60	-----	-----	-----	4.49	-----	-----	-----	4.80	-----

¹ Each liter of nutrient solution contained the following ingredients: Carbon compound, 50.0 gm.; NH₄NO₃, 2.06 gm.; KH₂PO₄, 0.55 gm.; MgSO₄·7H₂O, 0.25 gm.; iron, 0.30 mg.; zinc, 0.20 mg.; copper, 0.05 mg.; manganese, 0.025 mg.; molybdenum, 0.02 mg.; gallium, 0.02 mg.; and scandium, 0.04 mg.

² Sporulation was estimated on a scale ranging from 0 (= sterility) to 10 (= maximum sporulation); color of spores is indicated in all experiments by abbreviations of the words white, yellow, tan, brown, and black.

³ Maximum individual yield.

⁴ Coefficient of utilization, or yield per 100 gm. of sucrose.

⁵ Initial acidity of the nutrient solution.

An attempt to obtain improved results with the trace elements was made through the addition of supplementary sources of nitrogen displaying marked growth-promoting properties. These substances were selected on the basis of the comparative tests with organic compounds mentioned in the description of experimental procedures. The results of special tests with the most effective of these compounds are also given in table 1. Witte peptone proved most effective in supplementing the molecular deficiencies of glycerol. Bacto-peptone and sodium iron chlorophyllin were somewhat less effective. Lysine was least effective of the six organic nitrogen materials specially tested. Results with trace elements showed no improvement despite the appreciable increases in growth brought about through the addition of supplementary sources of nitrogen to the nutrient solution containing glycerol. The assumption is that the increase in mineral impurities brought about through their addition to the nutrient solution more than compensated for the beneficial action of increased yields for demonstrating trace-element deficiencies.

The specific nature of the molecular deficiencies with glycerol is not disclosed by the materials most effective in promoting growth. Particularly is this the case with Witte peptone and Bacto-peptone, both of which comprise an unknown mixture of protein-decomposition products, accessory factors, and inorganic salts (17). Sodium iron chlorophyllin, proline, and hemoglobin, however, are characterized by the presence of the pyrrole ring in their molecules. This ring or its tetrahydro derivative (pyrrolidine) is also without doubt present in Witte peptone and Bacto-peptone. Hammett (3) has claimed that the pyrrolidone group plays a special part in differentiation of the cell. Sodium magnesium chlorophyllin was not quite so effective in increasing the yield as was the iron salt. Miscellaneous tests with yeast (*Saccharomyces ellipsoideus*, A. T. C. C. No. 4097) indicated the magnesium salt to be more effective than the iron salt, especially in conjunction with *D*-inositol. Yield of yeast under these conditions was increased from 0.3 mg. to 4.8 mg. after 4 days' growth at 25° C. This compares favorably with a maximum yield of 18.4 mg. per culture of 50 ml. in a 5-percent sucrose solution containing 260 mg. of yeast extract per liter.

Similar tests of the growth-promoting properties of carbon compounds not containing nitrogen were also made and are given in table 2. These materials were relatively ineffective in comparison with the nitrogen compounds and were therefore supplied at a concentration of 1,000 mg. per liter instead of 20 mg. per liter. Sucrose seemed to be slightly more effective in promoting growth.

Sucrose was tested also at a concentration of 20,000 mg. per liter in order to demonstrate that the quantities of trace elements supplied to the fungus were ample for maximum growth with glycerol. This was considered advisable, since the quantities added had been determined to be the necessary minima with sucrose. Moreover, the possibility existed that glycerol might render the trace elements unavailable through formation of complex ions in which they were present in un-ionized form. This did not prove to be the case, however, since yield was maximum. The fact that yield did not exceed maximum in experiment 2 is due, of course, to the presence of only sufficient quantities of mineral constituents to give maximum yield.

TABLE 2.—Effects of a deficiency of trace elements on the growth of *Aspergillus niger* for 4 days at 35° C. with glycerol supplemented with other carbon compounds¹

Element omitted	Glycerol with indicated carbon compound (grams per liter)											
	Sucrose, 1 gm.				Sucrose, 20 gm.				Citric acid, 1 gm.			
	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe	134.3	17.97	2.61	6, bl	194.5	18.85	2.12	4, bl	75.6	17.15	2.41	6, bl
Zn	350.4	46.88	1.80	8, bl	561.6	54.43	1.79	10, bl	146.1	33.13	2.17	6, bl
Cu	789.5	105.63	2.26	6, t	1,213.9	117.64	3.07	10, t	245.1	55.57	1.94	6, bl
Mn	726.5	97.20	2.06	4, bl	1,182.0	114.55	2.26	1, bl	339.9	77.07	1.91	2, bl
Mo	718.4	96.12	2.08	8, bl	1,046.5	101.41	3.65	10, bl	435.8	98.81	1.86	8, bl
Ga	749.2	100.24	2.22	8, bl	1,036.1	100.41	3.92	10, bl	607.8	137.82	2.17	8, bl
Se	768.6	102.83	2.23	8, bl	1,032.1	100.02	3.96	10, bl	244.2	55.37	2.03	6, bl
None	747.4	100.00	2.18	8, bl	1,031.9	100.00	3.81	10, bl	441.0	100.00	2.01	6, bl
Max. ³	823.1	-----	-----	-----	1,213.9	-----	-----	-----	651.0	-----	-----	-----
C. U. ⁴	-----	32.92	-----	-----	-----	48.56	-----	-----	-----	26.04	-----	-----
pH ⁵	-----	-----	4.58	-----	-----	-----	4.70	-----	-----	-----	2.90	-----

Element omitted	Glycerol with indicated carbon compound (grams per liter)—Continued											
	Glycolic acid, 1 gm.				Pyruvic acid, 1 gm.				Glyceric acid, 1 gm.			
	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Sporulation ²
Fe	141.8	55.23	2.03	1, bl	70.1	22.06	2.21	2, bl	315.8	93.24	1.86	6, bl
Zn	246.8	96.14	1.95	2, bl	287.2	90.40	1.81	2, bl	109.0	32.18	2.04	10, bl
Cu	216.1	84.18	1.96	2, bl	257.4	81.02	1.84	2, bl	329.7	97.34	1.85	6, bl
Mn	702.3	273.59	3.24	0	603.2	189.86	2.36	0	676.1	199.62	2.38	4, bl
Mo	221.0	86.09	1.76	2, bl	257.4	81.02	1.83	2, bl	364.8	107.71	1.83	6, bl
Ga	233.2	90.84	1.92	2, bl	577.8	181.86	2.77	2, bl	368.8	114.20	1.86	6, bl
Se	267.0	104.02	1.92	3, bl	345.7	108.81	1.96	2, bl	206.7	61.03	2.06	6, bl
None	256.7	100.00	2.03	2, bl	317.7	100.00	1.92	2, bl	338.7	100.00	1.86	6, bl
Max. ³	702.3	-----	-----	-----	603.2	-----	-----	-----	711.7	-----	-----	-----
C. U. ⁴	-----	28.09	-----	-----	-----	24.13	-----	-----	-----	28.47	-----	-----
pH ⁵	-----	-----	2.99	-----	-----	-----	2.55	-----	-----	-----	3.07	-----

¹ See footnote 1, table 1.² See footnote 2, table 1.³ See footnote 3, table 1.⁴ See footnote 4, table 1.⁵ See footnote 5, table 1.

Attention is also directed to the results of deficiency tests with scandium in solution supplemented with citric acid and glyceric acid, giving yields of 55.35 and 61.05 percent of maximum respectively. The reason for these responses is unknown but it might be associated with the fact that these acids may be products of metabolism of this fungus. Results of deficiency tests with other elements and in other solutions were uniformly poorer than those usually obtained with sucrose. The sucrose, however, was of an especially high standard of purity (0.0014 percent ash).

GROWTH WITH SOURCES OF CARBON OTHER THAN GLYCEROL

Tests of trace-element requirements were also extended to include glucose, fructose, mannose, galactose, sorbose,³ mannitol, and lactose. The data for all but the last two are tabulated in table 3. Mannitol and lactose have been omitted because practically no growth of the

³ The sorbose used was obtained through the courtesy of the Bureau of Chemistry and Soils, U. S. Department of Agriculture. It had been produced through the fermentation of *d*-sorbitol by *Bacterium xylinum* and was considered better than 99 percent pure.

TABLE 3.—Effects of a deficiency of trace elements on the growth of *Aspergillus niger* for 4 days at 35° C. with different carbon compounds¹

Element omitted	Sucrose				d-Glucose				d-Fructose				d-Mannose			
	Yield per 2.5 gm. sucrose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. glucose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. fructose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. mannose	Proportion of maximum yield	Acidity at harvest	Sporulation ²
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe.....	57.3	6.54	2.46	2, bl	478.3	43.31	1.80	4, bl	1,009.0	95.57	1.95	7, bl	707.3	71.93	2.53	2, bl
Zn.....	32.9	3.75	2.97	2, bl	73.9	6.69	2.57	6, bl	90.9	8.61	2.49	10, bl	24.4	2.69	2.98	4, bl
Cu.....	688.2	78.52	2.41	1, bl	1,111.1	100.61	2.90	4, br	1,009.6	95.63	2.65	6, br	805.5	88.75	3.05	2, bl
Mn.....	791.9	90.35	2.11	2, bl	1,101.3	99.72	1.94	2, bl	1,045.3	99.01	2.34	10, bl	1,091.7	113.67	3.44	4, bl
Mo.....	440.4	50.25	1.74	2, bl	806.1	72.99	1.92	4, bl	873.1	82.69	2.30	6, bl	463.5	57.07	1.89	2, bl
Ga.....	834.1	95.16	2.44	6, bl	1,150.6	104.18	2.56	8, bl	1,058.1	100.22	2.38	10, bl	836.6	92.17	3.16	2, bl
Se.....	838.4	95.65	2.48	6, bl	1,148.3	103.97	2.80	8, bl	1,053.2	99.75	2.54	10, bl	878.7	96.81	3.50	2, bl
None.....	876.5	100.00	2.46	6, bl	1,104.4	100.00	2.76	10, bl	1,055.8	100.00	2.44	10, bl	907.6	100.00	3.62	2, bl
Max. ³	957.7				1,150.6				1,076.3				1,081.7			
C, U. ⁴		38.31				46.02				43.05				41.27		
pH ⁵			6.91				7.15								6.76	
Element omitted	d-Galactose ¹				L-Sorbose				L-Sorbose ¹							
	Yield per 2.5 gm. galactose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. sorbose	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. sorbose	Proportion of maximum yield	Acidity at harvest	Sporulation ²				
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH					
Fe.....	60.8	101.84	2.55	0	730.2	93.27	3.15	2, bl	903.8	92.86	4.23	2, bl				
Zn.....	60.6	101.51	2.54	0	594.9	75.98	2.27	2, bl	923.2	94.46	3.88	7, bl				
Cu.....	58.3	97.65	2.55	0	762.1	97.34	2.76	2, bl	968.3	98.46	4.25	6, bl				
Mn.....	56.6	94.81	2.53	0	860.1	109.86	3.70	2, bl	100.51	100.51	4.20	0				
Mo.....	58.3	97.65	2.52	0	512.9	65.51	2.19	2, bl	497.6	51.42	2.35	1, bl				
Ga.....	61.0	102.17	2.53	0	788.5	100.72	3.14	2, bl	868.5	98.48	3.86	6, bl				
Se.....	59.5	99.66	2.52	0	808.1	103.22	3.12	2, bl	947.8	97.38	4.35	6, bl				
None.....	59.7	100.00	2.52	0	782.9	100.00	2.87	2, bl	973.3	100.00	4.15	6, bl				
Max. ³					860.1				978.2							
C, U. ⁴		2.44				34.40				39.13						
pH ⁵			3.93				6.82				4.68					

¹ See footnote 1, table 1.

² See footnote 2, table 1.

³ See footnote 3, table 1.

⁴ See footnote 4, table 1.

⁵ See footnote 5, table 1.

¹ See footnote 1, table 1.

² See footnote 2, table 1.

³ See footnote 3, table 1.

⁴ See footnote 4, table 1.

⁵ See footnote 5, table 1.

¹ See footnote 1, table 1. ² See footnote 2, table 1.³ See footnote 3, table 1.⁴ See footnote 4, table 1.⁵ See footnote 5, table 1.

fungus occurred with these sources of carbon. Little doubt exists that the trace elements determined to be necessary with sucrose are also necessary with these other sources of carbon. Their degree of freedom from trace-element impurities was not sufficiently great to disclose whether special requirements do or do not exist. Repetition of these experiments at a higher level of purity would, therefore, be advisable.

Sucrose, glucose, fructose, mannose, and sorbose seemed equally effective for the nutrition of *Aspergillus niger*. Galactose was quite inadequate, however. The results with *l*-sorbose may need revision, since the responses with this source of carbon varied somewhat more in successive experiments than did those with the other carbon compounds.

The supplementary effect of traces of organic nitrogen compounds on growth with glycerol (table 1) appeared to offer a possible explanation for the relative unavailability of lactose, mannitol, and galactose, as well as glycerol. This interpretation was that substitutions of a poor carbon source for sucrose resulted in the induction of accessory-substance and amino acid requirements for supplementing the deficiency in necessary molecular configurations of the poor carbon source. The tests with organic supplements for glycerol were therefore continued further. The first two experiments of table 4 show the final results of these tests.

In the first experiment of table 4, the addition of 20 mg. per liter each of sodium iron chlorophyllin and β -phenylalanine increased the yield with glycerol to 906.1 mg., and the further addition of *l*-tyrosine increased the maximum yield to 961.6 mg. in experiment 2. That is to say, a total of 1 mg. each of these supplementary or accessory factors per culture sufficed to increase the yield from a maximum of 269.4 mg. (experiment 1, table 1) to one of 961.6 mg. It is logical to assume that continued tests for effective supplements would have resulted in a yield fully equal to that obtainable with sucrose, etc. Further investigations will, it is believed, lead to the discovery of more effective supplementary factors.

The results of similar studies with lactose are given in the third experiment of table 4. Sodium iron chlorophyllin again proved most effective, and sucrose was next in promoting growth. The other organic compounds tested proved ineffective, presumably because of the limited number available for trial. Results with galactose were even poorer at the supplementary level (20 mg. per liter) used. The poor results with lactose and galactose are attributed wholly to lack of addition of the appropriate compound to supplement the molecular deficiencies of these two compounds. This is not surprising, since less than 100 compounds were available for trial. The data in table 5 afford convincing proof that lactose and galactose are assimilable by *Aspergillus*, under appropriate conditions, also.

The last two experiments of table 4 illustrate the results obtained with mannitol when supplemented with traces of organic nitrogen. Sodium iron chlorophyllin again proved most effective, followed by *d*-glutamic acid, and *dl*- α -alanine. The action of these substances when the organism is grown on mannitol cannot be regarded as evidence for the requirement of accessory growth factors. Maximum yields are reached in 7 days by *Aspergillus* without their addition (19).

TABLE 4.—Effects of a deficiency of trace elements on the growth of *Aspergillus niger* for 4 days at 35° C. with glycerol, lactose, and mannitol in the presence of supplementary compounds of organic nitrogen¹

Element omitted	Glycerol with 20 mg. per liter of sodium iron chlorophyllin and β -phenylalanine				Glycerol with 20 mg. per liter of sodium iron chlorophyllin, β -phenylalanine, and β -tyrosine				Lactose with 20 mg. per liter of sodium iron chlorophyllin and 1.0 gm. per liter of sucrose				Mannitol with 20 mg. per liter of sodium iron chlorophyllin and α -glutamic acid				Mannitol with 20 mg. per liter of sodium iron chlorophyllin, α -glutamic acid, and α -alanine			
	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Spore-lation ²	Yield per 2.5 gm. glycerol	Proportion of maximum yield	Acidity at harvest	Spore-lation ²	Yield per 2.5 gm. lactose	Proportion of maximum yield	Acidity at harvest	Spore-lation ²	Yield per 2.5 gm. mannitol	Proportion of maximum yield	Acidity at harvest	Spore-lation ²	Yield per 2.5 gm. mannitol	Proportion of maximum yield	Acidity at harvest	Spore-lation ²
	Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe	791.4	93.81	2.33	6, t	814.2	85.81	2.59	4, br	284.1	95.37	1.82	10, bl	662.1	84.69	2.40	6, bl	718.5	84.33	2.83	4, bl
Zn	478.2	56.69	1.70	8, br	532.1	56.08	1.78	8, bl	223.4	75.00	1.82	10, bl	105.1	13.44	2.43	8, bl	156.4	18.36	2.81	8, bl
Cu	635.3	75.31	2.18	4, v	634.5	66.87	2.20	2, br	289.4	97.15	1.79	10, bl	365.9	46.80	2.07	4, bl	302.2	35.46	1.83	4, bl
Mn	458.6	54.37	1.78	6, bl	499.7	52.66	1.83	4, bl	298.3	100.14	1.78	10, bl	265.7	33.99	1.86	8, bl	374.1	43.90	1.85	8, bl
Mo	588.4	69.74	1.73	8, t	637.2	67.15	1.83	4, br	295.6	99.22	1.79	10, bl	473.8	60.62	1.93	6, bl	482.1	56.58	1.93	6, bl
Ca	841.7	99.77	2.93	6, t	911.9	96.11	3.16	4, br	286.3	96.20	1.79	10, bl	796.0	78.65	2.50	6, bl	742.8	87.18	3.20	6, bl
Se	847.5	100.46	2.73	6, t	924.9	97.48	3.06	6, br	286.6	96.20	1.79	10, bl	796.0	101.82	2.98	6, bl	710.2	83.35	3.62	6, bl
None	843.6	100.00	2.45	8, t	948.8	100.00	3.24	6, br	297.9	100.00	1.79	10, bl	781.7	100.00	2.69	6, bl	852.1	100.00	3.38	6, bl
Max. ³	906.1				961.6				301.6				796.0				935.6			
C, U, ⁴		36.24	5.08			38.46				12.06	3.93			31.84	4.62			37.42	4.62	
pH ⁵							3.86													

¹ See footnote 1, table 1.² See footnote 2, table 1.³ See footnote 3, table 1.⁴ See footnote 4, table 1.⁵ See footnote 5, table 1.

This would appear conclusive evidence that the fungus is entirely capable of their synthesis, though not perhaps at a rate as high as that at which they can be utilized.

The effects of trace-element deficiencies in the experiments with supplementary compounds were much the same as in those previously discussed. It appears probable that iron, zinc, copper, manganese, molybdenum, and gallium are required under all conditions.

EFFECT OF ADMIXTURE OF CARBON COMPOUNDS ON ASSIMILABILITY

That the effect of chlorophyllin and amino acids is actually to supplement deficiencies in molecular configuration is rendered clearer by the results given in table 5. These show the yields obtained with mannitol, glycerol, lactose, and galactose both singly and in mixture. All four of these poor carbon sources proved available for growth on proper admixture. The results with mannitol and galactose proved exceptionally interesting in view of the failure to find a suitable accessory for the latter in the previous experiments. Though the yield with mannitol was only 34.3 mg. and that with galactose 27.9 mg., the yield with a mixture of these compounds was 392.6 mg. Supplementation of mannitol with lactose also improved assimilation. It would be interesting to learn the relative assimilability of these sources in mixtures of even greater complexity and in the presence of supplementary nitrogen compounds.

TABLE 5.—Effect of equal admixtures (total of 2.5 gm. per culture) of poor carbon sources on yields of *Aspergillus niger*¹

Carbon compound	Compound used per culture	Yield	Mixture (2.5 gm. per culture)	Yield	
				Computed	Found
	Grams	Mg.		Mg.	Mg.
<i>d</i> -Mannitol.....	1.25	4.5	<i>d</i> -Mannitol+glycerol.....	231.2	545.1
Glycerol.....	1.25	226.7	<i>d</i> -Mannitol+ <i>d</i> -lactose.....	21.4	233.6
<i>d</i> -Lactose.....	1.25	16.9	<i>d</i> -Mannitol+ <i>d</i> -galactose.....	21.5	392.6
<i>d</i> -Galactose.....	1.25	17.0	Glycerol+ <i>d</i> -lactose.....	243.6	458.3
<i>d</i> -Mannitol.....	2.5	34.3	Glycerol+ <i>d</i> -galactose.....	243.7	154.7
Glycerol.....	2.5	349.0	<i>d</i> -Lactose+ <i>d</i> -galactose.....	23.9	16.7
<i>d</i> -Lactose.....	2.5	74.6			
<i>d</i> -Galactose.....	2.5	27.9			

¹ See footnote 1, table 1, for quantities of mineral constituents employed.

Experimental data showing the effect of trace-element deficiencies on growth and development with mixtures of inadequate carbon sources have been tabulated in table 6. Omission of any one of the trace elements appears to result in a slight but definite decrease in yield. With compounds of higher purity the effects of these omissions would have been enhanced, it is believed. Nonaddition of scandium also brought about a decrease in yield of a magnitude too great to be attributed to normal experimental variations.

Though a continuation of these experiments with a nutrient solution purified with calcium carbonate would probably have led to greater differences with trace-element deficiency, this method was not employed. Neither were any experiments carried out to ascertain

the effects of admixture of more than two sources of carbon, nor of a combination of this procedure with tests of supplementation with various organic nitrogen compounds.

TABLE 6.—Effect of deficiencies in trace elements on utilization of carbon compounds in admixture by *Aspergillus niger* grown for 4 days at 35° C.¹

Element omitted	Mixture of <i>d</i> -mannitol and <i>d</i> -galactose				Mixture of <i>d</i> -lactose and glycerol			
	Yield per 2.5 gm. of carbon sources	Proportion of maximum yield	Acidity at harvest	Sporulation ²	Yield per 2.5 gm. of carbon sources	Proportion of maximum yield	Acidity at harvest	Sporulation ²
	Milli-grams	Percent	pH		Milli-grams	Percent	pH	
Fe.....	301.7	80.51	1.74	0	398.2	69.90	1.88	2, bl
Zn.....	344.2	91.86	1.74	0	328.2	57.61	1.74	3, bl
Cu.....	353.4	94.31	1.87	0	497.6	87.36	2.03	8, bl
Mn.....	399.4	106.59	1.94	0	533.8	93.72	2.14	2, bl
Mo.....	247.1	65.94	1.80	0	515.4	90.49	2.10	6, bl
Ga.....	351.1	93.70	1.80	0	493.2	86.59	2.07	2, bl
Sc.....	316.4	84.44	1.80	0	508.7	89.30	2.09	2, bl
None.....	374.7	100.00	1.88	0	569.6	100.00	2.16	2, bl
Max. ³	399.4	-----	-----	-----	600.4	-----	-----	-----
C. U. ⁴	-----	15.98	-----	-----	-----	24.02	-----	-----
pH ⁵	-----	-----	3.81	-----	-----	-----	3.75	-----

¹ See footnote 1, table 1.

² See footnote 2, table 1.

³ See footnote 3, table 1.

⁴ See footnote 4, table 1.

⁵ See footnote 5, table 1.

EFFECT OF AN EXTERNAL SUPPLY OF METABOLIC PRODUCTS

Growth of *Aspergillus niger* in the dibasic optimum solution with sucrose is equal to or greater than that of other hexoses, being approximately 1,150 mg. dry weight per culture of 50 cc. (2.5 gm. sucrose) after 4 days at 35° C. The average rate of growth, therefore, is almost exactly 12 mg. per hour, or 1.04 percent per hour of the maximum yield in a solution in which the organism is compelled to synthesize all its metabolic products from sucrose and inorganic nitrogen.

The experimental data in table 7 show the effect of the metabolites in yeast extract on the growth of *Aspergillus* when substituted in part for sucrose. The strain of *Aspergillus* employed in this experiment does not require the presence of accessory growth factors. The mineral constituents were present in optimum amount, only sucrose being decreased from 50 gm. per liter to 40 gm. In every case the yield with yeast extract was greater than that with an equivalent quantity of sucrose over and above the basic 40 gm. per liter. A yield of 1,214 mg. was reached in 3 days with 40 gm. of sucrose and 8 gm. of yeast extract per liter. The average rate of growth was 17 mg. per hour, or 1.4 percent per hour of maximum yield. The yield with 50 gm. of sucrose per liter had reached a value of only 1,043.4 mg. after 4 days of growth. The beneficial effects of yeast extract are especially striking during the early stages of growth. The average rate of growth was 11 mg. per hour, or 1.05 percent per hour of maximum yield.

TABLE 7.—Increased efficiency of carbon utilization by *Aspergillus niger* at 35° C. through partial replacement of sucrose by yeast extract ¹

Growth period	Substance added	Yields obtained with indicated number of grams per liter of substance added to solution containing 40 gm. of sucrose per liter					
		0	2	4	6	8	10
Days		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
2	{ Sucrose.....		218.6	165.7	172.2	159.7	229.5
	{ Yeast extract.....		859.1	981.2	1,036.3	1,063.0	1,065.8
3	{ Sucrose.....	689.2	714.0	702.5	743.8	761.9	749.7
	{ Yeast extract.....		1,083.1	1,125.6	1,172.8	1,214.0	1,224.3
4	{ Sucrose.....	894.6	856.2	941.7	997.4	1,048.4	1,043.4
	{ Yeast extract.....		971.3	1,021.7	1,083.0	1,103.5	1,140.5

¹ See footnote 1, table 3, except that sucrose was employed at 40 gm. per liter.² Sporulation had not begun in the sucrose cultures, whereas the yeast cultures were black with spores.

DISCUSSION

Nothing requiring special comment is presented by the results on trace-element requirements of the fungus, except in the case of scandium. Scandium proved quite beneficial to growth of the organism when glycerol was supplied as a source of carbon. Its nonaddition with other sources of carbon was quite without effect, however, and differs in this respect from the results obtained with the other trace elements. Iron, zinc, copper, manganese, molybdenum, and gallium usually gave indications of their essentiality for metabolism with all sources of carbon, even though the differences were sometimes slight. This might have been anticipated in view of the lesser freedom from mineral impurities of these carbon compounds as compared with sucrose. A possible relation may exist, therefore, between the low molecular weight of glycerol, necessitating as it does a greater degree of metabolic molecular condensations, and the ability to demonstrate its action on the fungus. The specific action of scandium with glycerol was subjected to repeated tests with a large number of the chemical elements, and there would seem to be little doubt that it exists.

Growth with reagent glycerol in the absence of scandium amounted to only 107.4 mg., and decreased to 57.5 mg. when the glycerol was redistilled in a pyrex glass still. Purification with calcium carbonate of the nutrient solution containing redistilled glycerol caused a further decrease in yield to 21.7 mg. The yields in the presence of scandium were 269.4 mg., 54.6 mg., and 57.9 mg. respectively. Addition of even highly purified sucrose to the medium in small quantity (1 gm. per liter) eliminated the effect of scandium. Other organic substances tried behaved similarly, except citric acid and glyceric acid.

Growth of the fungus proved optimum on sucrose, glucose, fructose, mannose, and sorbose. Yields with lactose, galactose, and mannitol were very poor. The effect of the time element on this response is known for mannitol (19) only, and indicates that maximum yield requires 7 days for its attainment as compared to 4 days with sucrose. It appears to differ in this respect from glycerol, lactose, and galactose.

The experimental data on the carbon nutrition of the organism obtained under these circumstances have proved to be of considerable interest. Briefly, they comprised evidence that poor sources of carbon (*d*-mannitol, *d*-galactose, *d*-lactose, glycerol) gave marked

increases in growth on admixture. It was found further that substitution of a poor carbon source for sucrose resulted in the induction of special amino acid and accessory-factor requirements.

The response of the organism when grown with glycerol or mannitol differed greatly from that with lactose and galactose upon the addition of small quantities of other organic compounds. The substances that were tested have been listed in a previous paper (17). Yields with glycerol were practically maximum in the presence of traces of sodium iron chlorophyllin, β -phenylalanine, and *l*-tyrosine. The same was true for mannitol in the presence of traces of sodium iron chlorophyllin, *d*-glutamic acid, and *dl*- α -alanine. A distinct though slight effect was produced by sodium iron chlorophyllin with lactose, though other nitrogen compounds proved ineffective. Traces of none of the compounds available for test proved effective in aiding the growth of the fungus on galactose.

The effects obtained by the addition of traces of organic nitrogen compounds to glycerol and mannitol are interpreted as due to the induction of special nitrogen requirements. Glycerol and mannitol are probably poor sources of carbon because they do not furnish the specific molecular groups required by the organism in the synthesis of organic nitrogen. This view is in agreement with the results obtained with carbon compounds in admixture.

Perhaps the most interesting feature encountered was the discovery that rate of growth with an optimum solution containing sucrose and inorganic nitrogen could be greatly increased by the addition of yeast extract. The acceleration in growth is presumed to be brought about by the presence in yeast extract of many of the metabolic products that *Aspergillus* usually has to synthesize from sucrose and inorganic nitrogen. That this is the correct interpretation is rendered more probable by the accompanying slight but definite increases in efficiency of carbon utilization with yeast extract. The identification and production in a high state of purity of the metabolic products responsible for the acceleration of growth would be highly desirable in connection with studies dealing with the functions of the trace elements.

SUMMARY

Trace-element and carbon-compound requirements were studied with cultures of *Aspergillus niger* Van Tiegh. grown for 4 days at 35° C. The results on trace-element needs with the sources of carbon used were poorer than those obtained with sucrose of exceptional purity.

Iron, zinc, copper, manganese, molybdenum, and gallium would appear to be necessary whatever the source of carbon. Scandium exhibited biological specificity with the fungus when grown on glycerol, the yield being doubled.

Sucrose, *d*-glucose, *d*-fructose, *d*-mannose, and *l*-sorbose proved to be excellent sources of carbon and equally effective for nutrition, whereas glycerol, *d*-mannitol, *d*-lactose, and *d*-galactose proved quite ineffective.

Admixture of these carbon compounds, incapable of assimilation when available as sole carbon sources, was found markedly to increase their assimilability. Increased assimilability on admixture was interpreted on the basis of mutual supplementation of compounds deficient in essential molecular configurations.

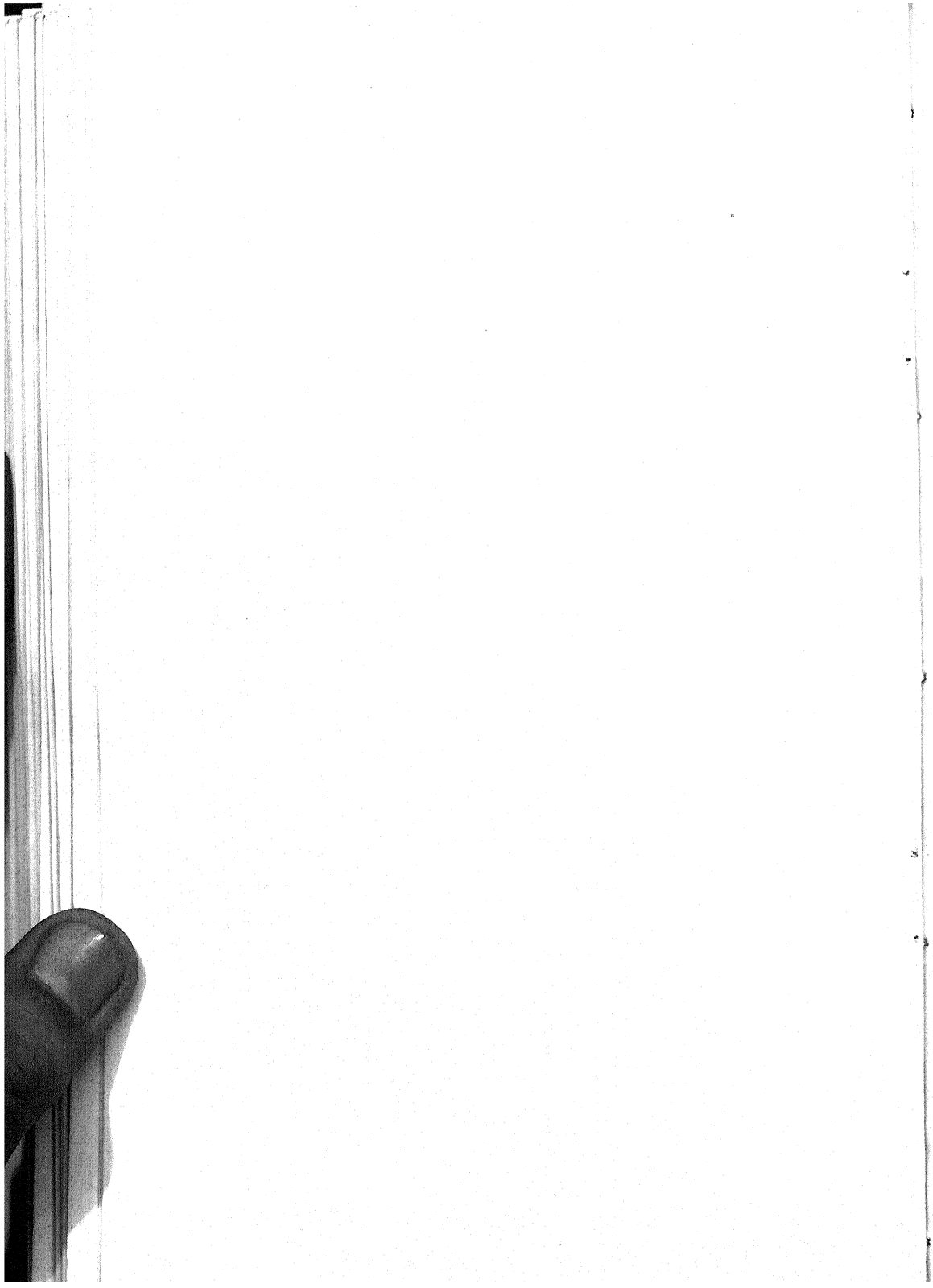
Growth with glycerol and mannitol could also reach maximum when these compounds were supplemented with traces of sodium iron chlorophyllin and specific amino acids. The beneficial effects of traces of supplementary nitrogen compounds on carbon sources of low assimilability is attributed to the induction of special requirements for supplementation of deficiencies in molecular configuration. They are not considered due to the necessity for accessory growth factors, since the organism is fully capable of maximum growth with inorganic nitrogen and sucrose.

Tests with yeast extract indicated that a supply of metabolites on a sucrose medium caused acceleration in growth, under otherwise optimum conditions, not attributable to accessory-factor requirements. These effects of organic nitrogen supplementation were due to the existence of limiting factors in functional processes of the fungus and not to absence of the function.

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AMINO ACIDS IN THE CORN KERNEL ¹

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INTRODUCTION

A knowledge of the amino acid composition of individual staple foods used in human and animal dietary is of value to the nutritional worker as well as to the agriculturist. The classification of amino acids as dispensable and indispensable emphasizes the protein quality rather than the quantity requirement of protein in the daily diet. We do not eat or feed purified proteins of known amino acid composition. From the nutritional standpoint, staple foods are generally consumed in combinations in order to correct or supplement certain deficiencies in amino acids, vitamins, minerals, etc., and thus avoid the use of concentrates. The present study in the series of amino acids in staple foods is a further attempt to furnish data on corn (*Zea mays* L.).

Pellagra has been found to occur in sections where the population is poorly nourished and lives chiefly on corn. This observation suggested the nutritional origin of the disease, and the low quality of corn proteins was suspected as a contributing factor to the malnourished state of pellagrins. Osborne and Clapp (5)² analyzed zein, the alcohol-soluble corn protein, and noted the absence of tryptophane and lysine, both of which are nutritionally indispensable amino acids. In the corn glutelin, the author has found 0.516 percent of tryptophane (1); thus the absence of tryptophane in the zein is largely, if not wholly, corrected when whole corn is eaten.

EXPERIMENTAL METHODS

The method for determining amino acids has been described in two previous publications (2, 3). Certain changes have been introduced, which are given in the following paragraph, to suit the analytical procedure to the property of the protein material present in the corn kernel.

A white corn and a yellow corn³ were selected for analysis. The cleaned and selected whole corn kernels were ground to a fine meal and stored at a temperature slightly below freezing. Duplicate samples (air-dry) of 25 gm. each were used for analysis. Ether extraction to remove fatty substances was omitted, because it lowers the solubility of protein in a 1-percent salt solution (2). The finely ground corn meal was, therefore, extracted with 100 cc. of precooled 1-percent NaCl solution. Three of these salt extractions of 1-hour duration at 6°-8° C. were applied for removal of the water- and salt-

¹ Received for publication May 15, 1939. This is the third in a series of papers on amino acids in staple foods.

² Italic numbers in parentheses refer to Literature Cited, p. 768.

³ The author is indebted to M. T. Jenkins, of the Bureau of Plant Industry, and to H. C. Fellows, of the Bureau of Agricultural Economics, U. S. Department of Agriculture, for supplying and grinding the corn samples.

soluble proteins. Then the residue was extracted twice with 80-percent alcohol in 100-cc. portions at room temperature. One of these alcoholic extractions was of 3 hours' duration, and another lasted overnight. The third alcoholic extraction was carried out at 56°–58° C. for only one-half hour. The residue was cooled in a refrigerator, and the starch removed by the addition of a 21-percent HCl solution, using 200 cc. the first time at refrigeration temperature for 1 hour and stirring occasionally to facilitate the dispersion of starch. The acid extract separates well from residue "R" by centrifugation. By the addition of an equal volume of 95-percent alcohol to the acid extract the starch precipitates. The extract was then centrifuged and the supernatant liquid was decanted. The decanted liquid was then evaporated to a small volume and added to the 1-percent NaCl and 80-percent alcohol extracts. The residue "R" was extracted twice more with 21-percent HCl to remove all starch, 50 to 100 cc. of acid being used each time. These acid extracts do not separate clearly by centrifugation and the slightly turbid supernatant liquid has to be filtered, preferably in the refrigerator. Since the second and third acid extracts contained only starch and an insignificant quantity of nitrogen, the filtrate was discarded. The precipitate from the filtrate, however, was removed and added to the starch-freed residue and hydrolyzed for 24 hours in 20-percent HCl. This hydrolysate, together with the hydrolysate of the combined salt, alcohol, and acid-alcohol extracts, was analyzed for the amino acids as described in previous publications (2, 3).

The distribution of nitrogen in the extracts already referred to was approximately the same in the white and yellow corn samples and together represented from 90 to 92 percent of the total nitrogen.

The salt- and alcohol-insoluble type of protein constituted the largest percentage (48 percent); the 80-percent alcohol-soluble protein, 27 percent; and the 1-percent NaCl solution, 16 percent. It should be noted that the distribution of nitrogen in the different extracts is comparable only when the several factors, such as fineness of ground particles of meal, the concentration of salt or alcoholic solution, the order of the extraction by the different solvents, and the varieties under investigation, are identical. An 80-percent alcoholic solution, for example, invariably removed more nitrogen than an 85-percent solution in the procedure just described.

In regard to the determinations of tryptophane⁴ and histidine, a few remarks are needed. By using a Bürker colorimeter, which is equipped with color compensation chambers, a decided improvement was observed in color matching by the May and Rose tryptophane method as modified by the author (1). The Ehrlich reagent was omitted from the samples used for color compensation, otherwise the technique was similar to that used for the standard and for the unknown samples. The color compensation for both standard and unknown solutions results in a more accurate color match. In regard to the histidine determination, it was found advantageous to decolorize the solution with Carboraffin before it was precipitated by Hopkin's mercuric sulfate reagent.

⁴ An error in calculation was discovered in the tryptophane percentages in the earlier papers (3, 6). To obtain correct values those given for wheat, bran, and shorts should be doubled.

EXPERIMENTAL DATA

Showalter and Carr (7) state that the proportion between zein and the other proteins varies according to the nitrogen content of the corn.

The nitrogen content of the two types of corn selected for this investigation differed little, as is shown in table 1; therefore a difference found in the amino acid composition might be significant in selecting one or the other on the basis of protein quality. The analytical results as shown in table 1, however, do not reveal any justification for preferring white corn to yellow corn with respect to amino acid composition. On a dietary regimen where the protein requirement is covered only by the corn proteins unsatisfactory growth of animals has been reported (4), not because of lack of any specific amino acid, but rather because of insufficient quantities of some of those indispensable ones considered herein. This conclusion is reached on the basis of Rose's figures representing the minimum quantities of indispensable amino acids required to support growth (6). Furthermore, in table 2, where the amino acids are expressed in quantities as obtained from 1 gm. of nitrogen of the whole corn kernel, the inferior quality of the corn protein as compared to casein is clearly demonstrated in respect to tryptophane and lysine. A judiciously selected mixed diet, however, should correct a poor nutritional state brought about by a diet in which corn is the chief source of protein.

TABLE 1.—Amino acid content and total nitrogen of two varieties of moisture-free corn grown in 1937

Place grown and variety of corn	Cystine	Tryptophane	Tyrosine	Arginine	Histidine	Lysine	Total nitrogen
	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Virginia: Boone County White.	0.096	0.047	0.703	0.212	0.089	0.107	1.71
Iowa: Black Yellow Dent.....	.095	.053	.700	.226	.109	.108	1.78

TABLE 2.—Protein quality of indicated amino acid obtained from 1 gm. of staple food nitrogen with casein for comparison

Staple food	Cystine	Tryptophane	Tyrosine	Arginine	Histidine	Lysine
	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>	<i>Milligrams</i>
White corn.....	56	28	441	124	52	63
Yellow corn.....	53	30	393	127	61	61
Casein.....	20	130	405	236	156	475

SUMMARY

The present study shows definitely that none of the indispensable amino acids considered herein are missing from the whole corn flour and that they are equally distributed in white and yellow corn. The amino acid composition of the whole corn kernel as found and as described in this paper when compared with that of casein shows that tryptophane and lysine are present at a lower level. The deficiency of tryptophane and lysine, which are absent in zein, apparently is not corrected sufficiently by the rest of the corn proteins. This conclusion, based on analytical findings, supports the general feeding practice of supplementation.

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EXPERIMENTAL TRANSMISSION OF BOVINE VENEREAL TRICHOMONIASIS¹

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INTRODUCTION

In spite of the fact that knowledge concerning bovine venereal trichomoniasis has been obtained only during the last 10 years, it does not follow that the disease is new. Probably it has existed for a long time. A review of the literature and of the salient facts concerning the disease was given in a previous paper by Rees.² That review showed that much of the work of previous investigators was epizootological and that there is need of long-term experiments to supplement existing data.

The specific objectives of the experiments recorded in this paper were as follows: (1) To observe the effects, on the fecundity of cows, of venereal trichomoniasis transmitted either by intravaginal inoculation of the parasite *Trichomonas foetus*, from bacteria-free and virus-free cultures, or by coitus with infected bulls; (2) to determine whether these female hosts would recover, and, in the event of recovery, whether they would be susceptible to reinfection; and (3) to obtain data on infection in the genital tract of the bull.

MATERIALS AND METHODS

In the experiments reported in this paper 10 heifers and 3 bulls were used. All were free from *Brucella abortus* infection and from tuberculosis, and at the beginning, from trichomoniasis also. Seven of the heifers, after an abortion or the birth of a calf, were again used for the experimental transmission of the disease. These animals were kept in $\frac{1}{2}$ -acre paddocks provided with adequate shelter and had access to a limited quantity of green feed and an ample quantity of hay, grain, and minerals.

The parasites (*Trichomonas foetus*) used for inoculating the females were freed from bacteria either by the method of Glaser and Coria³ or of Rees⁴ and cultivated in modified egg-Ringer-serum medium of Boeck and Drbohlav.⁵ Infection of the females was accomplished either by intravaginal inoculation of *T. foetus* or through coitus with an infected bull. Inoculation was performed with a glass cannula to which a rubber bulb was attached. About 15 cc. of Ringer's-serum fluid in which the trichomonads were growing was inoculated high up in the vagina near the os uteri. The infected bull used in the transmission experiments acquired the infection through coitus with a heifer that had been infected through inoculation. Regardless of the

¹ Received for publication July 11, 1939.

² REES, CHARLES W. BOVINE VENEREAL TRICHOMONIASIS. North Amer. Vet. 19 (4): 33-36, illus. 1938.

³ GLASER, R. W., and CORIA, A. PURIFICATION AND CULTURE OF TRICHOMONAS FOETUS (RIED-MÜLLER) FROM COWS. Amer. Jour. Hyg. 22: 221-226. 1935.

⁴ REES, CHARLES W. OBTAINING BACTERIA-FREE PURE LINES OF TRICHOMONAS FOETUS BY MEANS OF MICROISOLATION. Amer. Jour. Hyg. 26: 283-291, illus. 1937.

⁵ BOECK, WILLIAM C., and DRBOHLAV, JAROSLAV. THE CULTIVATION OF ENDAMOEBA HISTOLYTICA. Amer. Jour. Hyg. 5: 371-407, illus. 1925.

method used to infect the cattle, no bacteria or viruses were introduced into the animals.

To determine the onset and the duration of the infection the cattle were examined twice a week, beginning at the time of exposure. The method of examination was the same as that described previously by Rees.⁶

EXPERIMENTAL DATA

RESULTS WITH FEMALES

The experimental data on the females are recorded in table 1. The following significant points are shown: (1) Heifer 161 failed to calve and was positive for trichomonads for almost 2 years, during which time there were no heat periods. (2) Five other animals (Nos. 157, 158, 160, 163, and 174) when exposed as heifers, and Nos. 155 and 156 when exposed as cows, all became positive for *Trichomonas foetus*, some of them remaining positive for as long as 136 days. While positive these animals either had irregular heat periods or failed entirely to show signs of oestrus; no conceptions occurred. (3) Heifer 159 was a nonbreeder. (4) Heifer 162, the only infected heifer showing no delay in calving, became pregnant first and was exposed to the disease later; in this case the cervical seal appears to have kept the trichomonads out of the uterus. As a cow, this animal showed little resistance. (5) Four animals, Nos. 157, 158, 160, and 163, that had been infected as heifers and had lost the infection, became reinfected through coitus but were positive for only 1 to 22 days; they became pregnant without appreciable delay.

Table 1 shows (1) that more than half of all examinations were positive, the percentage being 58; (2) that some samples appearing negative on immediate examination were positive after incubation; and (3) that some samples positive on immediate examination were negative after incubation. It is evident, therefore, that a combination of both examinations yielded more positives than would have been detected if only one method had been relied upon. A fact not shown in the table is that in most of the cases there were periods when all examinations were positive, alternating with periods when most of them were negative. As a rule, there was a period of heat near the close of the period of positive examination and usually, when bred at that time, the animal became pregnant.

The following explanation is offered for the lack of data on the incubation period and the duration of the infection of heifer 163: This animal was intended as a control and was served on April 9, 1937, by bull 150 which was thought to be noninfected. The reason for regarding this bull as noninfected was that between August 20, 1936, and March 1, 1937, the animal had been examined 14 times and found to be negative for *Trichomonas foetus*. With several exceptions, the examinations were of material from three sources, namely, the seminal vesicles, the vasa deferentia, and the prepuce. Examinations were made at the time that the samples were obtained and also after incubation in vitro. However, the examinations made on June 23, 1937, 75 days after coitus of bull 150 with heifer 163, showed both the heifer and the bull to be positive for *T. foetus*. Since, prior to April 9, heifer 163 was virgin and free from *T. foetus* and from April 9 to June 23 she had not been exposed, she must have acquired the

⁶ See footnote 2.

infection from the bull on April 9, and therefore this bull must have been infected.

The reason for the lack of data on the method of exposure and length of the incubation period of heifer 161 is as follows: On May 18, when bull 150 served this heifer, it was not known that the bull was infected and consequently the heifer was inoculated, as shown in table 1. Therefore, infection may have been acquired through either coitus or inoculation or by both methods. For 9 months following service on May 18, heifer 161 was considered to be pregnant. However, on March 4, 1938, about a month after expected parturition, a rectal examination was made which led to a tentative diagnosis of pyometra. On March 7, by the use of all known precautions against sepsis, a cannula was placed through the cervical canal and 13½ quarts of pus was aspirated from the uterus. The pus contained leucocytes and trichomonads but was free from bacteria. On March 27, 7½ quarts of foul-smelling pus, which contained bacteria, was aspirated. From February 1938 to March 4, 1939, there were remittent vaginal discharges containing *T. foetus*.

The fetus of cow 158 that aborted on the two hundred and forty-fifth day of gestation weighed 42 pounds and was fully haired. Material from both fetus and placenta was free from trichomonads. Therefore, the abortion was probably not due to trichomoniasis.

RESULTS WITH BULLS

On October 6 and 7 and on November 27, 1936, bull 150 served heifer 157 after the latter had been exposed to trichomonad infection. On September 3 and October 7, 1936, and on January 14 and 21, 1937, he served heifer 160. Apparently during one of these services this bull became infected with *Trichomonas foetus*, although, as stated previously, a positive diagnosis was not made until June 23, 1937. These data show that *T. foetus* was transmitted from a heifer to a bull.

Table 1 shows that after the infection was acquired, bull 150 served each of eight females either as heifers or as cows, one or more times. He served a ninth female, both as a heifer and as a cow. Without exception transmission followed coitus regardless of whether the female in question had or had not been previously infected.

In eight cases following coitus the incubation periods were 12, 11, 14, 13, 11, 11, 6, and 11 days. The average period of incubation was slightly more than 11 days. In three animals infected by intravaginal inoculation the incubation periods were 2, 2, and 4 days.

In comparing the infection of the male bovine host with that of the female it will be noted that with one exception the latter recovered from the disease and exhibited resistance to reinfection. On the other hand, the male has been continuously infected for 20 months, and at present the infection appears to be permanent.

Trichomonad infection had no appreciable effect on the potency of this bull as evidenced by the undelayed conception of reinfected cows 157, 158, 160, and 163.

On October 2, 1936, and February 5, 1937, bull 149 served heifer 158. On October 7, 1936, he served heifer 160, and on February 9, 1937, heifer 157. In every case the service took place after the heifers had been exposed to *Trichomonas foetus*. To March 4, 1939, bull 149 has not become positive for trichomonads.

TABLE 1.—Results of experimental infection with *Trichomonas foetus* of 10 bovine animals

Animal designation	Method of exposure	Dates of transmission	Incubation period	Dates when first and last positive examinations occurred	Period during which examinations were positive for <i>T. foetus</i>			Examinations positive			Total examinations	Total examinations negative	Bull used		Date of coitus	Conception (+) or nonconception (-)	Interval between heat periods	Date of calving	Approximate time between first coitus and calving	Remarks
					Days	Period during which examinations were positive for <i>T. foetus</i>	Days	Immediate	After incubation	Total positive	No.	Examinations negative	No.	Infected (+) or noninfected (-)						
No. 155: As a heifer											149				Sept. 6, 1936	+	Days	June 21, 1937	Months 9	Control; not infected.
As a cow	Coitus	Sept. 25, 1937	13	{ Oct. 8, 1937, and Feb. 21, 1938.	{ 136	{ 4	{ 16	{ 16	{ 14	{ 30	{ 150 150 150 150	{ +	{ Sept. 25, 1937 Mar. 7, 1938 Mar. 7, 1938 Mar. 24, 1938	{ -	{ 164 7 48	{ -	{ 164 7 48	{ June 21, 1937 Feb. 14, 1939	{ 16½	{ Bull 150 positive sometime early in 1937. See text.
No. 156: As a heifer											149				Sept. 11, 1936	+	Days	June 11, 1937	9	Control; not infected.
As a cow	Coitus	Sept. 24, 1937	14	{ Oct. 8, 1937, and Feb. 21, 1938.	{ 136	{ 8	{ 5	{ 8	{ 22	{ 30	{ 150 150	{ +	{ Sept. 24, 1937 Jan. 31, 1938	{ -	{ 129	{ -	{ 129	{ Nov. 3, 1938	{ 13½	
No. 157: As a heifer	Inoculation	{ Sept. 14, 1936 Oct. 6, 1936 Oct. 12, 1936	{ (1)	{ Oct. 13, 1936, and Jan. 4, 1937.	{ 83	{ 16	{ 20	{ 20	{ 12	{ 32	{ 150 150 149	{ -	{ Oct. 6, 1936 Oct. 7, 1936 Nov. 27, 1936 Feb. 9, 1937	{ -	{ 51 74	{ -	{ 51 74	{ Nov. 12, 1937	{ 13	
As a cow	Coitus	{ Mar. 22, 1938 Apr. 4, 1938	{ 11	{ Apr. 2 and Apr. 22, 1938.	{ 20	{ 1	{ 5	{ 5	{ 8	{ 13	{ 150 150	{ +	{ Mar. 22, 1938 Apr. 4, 1938	{ -	{ 13	{ -	{ 13	{ Jan. 2, 1939	{ 9½	
No. 158: As a heifer	Inoculation	{ Oct. 2, 1936 Oct. 7, 1936	{ (1)	{ Oct. 8 and Dec. 30, 1936.	{ 83	{ 31	{ 30	{ 31	{ 2	{ 33	{ 149 149	{ -	{ Oct. 2, 1936 Feb. 5, 1937	{ -	{ 126	{ -	{ 126	{ Nov. 7, 1937	{ 13	
As a cow	Coitus	Apr. 20, 1938	6	Apr. 26, 1938	1	1	1	1	0	1	{ 150 150	{ +	{ Apr. 20, 1938 Apr. 29, 1938	{ -	{ 9	{ -	{ 9			
No. 159 (heifer)	Inoculation	Aug. 23, 1936	2	Aug. 24 and Sept. 14, 1936.	21	6	6	6	0	6										

{ Aborted Dec. 30, 1938; not caused by *T. foetus*.
Non breeder.
Slaughtered Dec. 9, 1936.

No. 160: As a heifer.	do.	do.	2	{ Aug. 24 and Dec. 30, 1936.	{ 128 }	12	12	12	32	{ 150 149 150 }	-	-	-	Sept. 3, 1936 Oct. 7, 1936 Jan. 14, 1937 Jan. 21, 1937 Mar. 13, 1938	-	-	-	{ 34 99 7 }	{ Oct. 24, 1937 Dec. 22, 1938 }	{ 13½ 9 }	Pyometra.	Pregnant when first exposed to infection.	{ 11 12½ 9 }	{ (Diagnosis delayed; see text.
As a cow	Coitus	Mar. 13, 1938	12	{ Mar. 25 and Apr. 16, 1938.	22	11	12	12	1	13	+	+	+		+	+	+							
No. 161 (heifer)	Coitus	May 18, 1937 { May 19, 1937 May 21, 1937 May 24, 1937 May 27, 1937 }	{ (1) }	{ June 1, 1937, and Mar. 4, 1939.	{ 641 }	59	69	69	61	130	+	+	+	May 18, 1937	+	+	+							
No. 162: As a heifer	do.	May 28, 1937	4	{ June 1 and July 9, 1937.	38	15	14	15	1	169	-	+	+	May 8, 1937	+	+	+							
As a cow	Coitus	Apr. 7, 1938	11	{ Apr. 18 and May 18, 1938.	{ 30 }	17	21	21	0	{ 150 150 }	+	+	+	{ Apr. 7, 1938 June 10, 1938 }	-	+	+	64	{ Mar. 13, 1939 }					
No. 163: As a heifer	do.	Apr. 9, 1937	(1)	{ June 23 and July 1, 1937.	{ (1) }	1	3	3	2	5	+	+	+	{ Apr. 9, 1937 July 2, 1937 July 19, 1937 July 26, 1938 }	-	+	+	84 17	{ Apr. 26, 1938 Apr. 30, 1939 }	{ 12½ 9 }				
As a cow	do.	July 26, 1938	11	{ Aug. 6 and Aug. 11, 1938.	5	2	2	2	2	4	+	+	+		-	+	+							
No. 174 (heifer)	do.	Sept. 13, 1937	11	{ Sept. 24 and Oct. 28, 1937.	{ 34 }	8	7	8	5	{ 150 169 }	+	+	+	Sept. 13, 1937 Jan. 27, 1938	-	+	+	136	{ Nov. 5, 1938 }	{ 13½ }				
Total						192	223	229	162	391														

1 Not known.

On January 27, 1938, bull 169 served heifer 174 after she had been positive for *Trichomonas foetus*; this bull has likewise shown no evidence of infection. These data appear to indicate that experimental infection of bulls may be difficult.

DISCUSSION

The data on 8 of the 15 infected cases in the female host serve to confirm and amplify those of previous investigators. The infections had one of the following consequences: (1) Failure of early conception and temporary loss of periodicity of oestrus followed by final overcoming of the infection and the restoration to a normal condition, or (2) conception followed by the death of the fetus in utero and the development of pyometra.

The data on the remaining seven cases bring new facts to light, as follows: After infection with *Trichomonas foetus* a heifer failed entirely to breed; however, additional data are needed to determine whether this failure was the result of infection or whether the animal was sexually undeveloped. In a pregnant bovine *T. foetus* established itself in the vagina but was apparently unable to reach the uterus through the cervical seal; as a cow, this animal did not show appreciable resistance to reinfection. Other females that contracted the disease as heifers either before or at the time of coitus showed, as cows, marked resistance to the disease; the infection did not appreciably delay conception.

The data give no indication of a difference in the course of the disease whether transmitted by coitus with an infected bull or by intravaginal inoculation with bacteria-free and virus-free cultures of *Trichomonas foetus*.

Data on the infection in the male bovine host show that after an incubation period of about 11 days a vaginal infection developed in every female served by an infected bull.

The resistance of cows to reinfection after having overcome attacks of the disease as heifers is the most encouraging aspect of the problem. From such resistant cows, calves may be sired by infected bulls without delay of conception or other consequences that usually follow the breeding of heifers and susceptible cows to infected bulls. Further work is needed, however, to determine the duration of the resistance in cows. Infection of heifers and cows is a matter of grave concern, since even in mild cases trichomonad infection lowers the fecundity of the herd. In severe cases, in which pyometra may develop, the fecundity of the female may be destroyed.

The most serious aspect of bovine venereal trichomoniasis is the infection of the bull, for the following reasons: (1) Once acquired, the infection appears to be permanent; (2) transmission by the bull may be expected to occur at every act of coitus, and even semen from an infected bull used for artificial insemination has been shown to result in transmission of the disease⁷; (3) no method of treatment for the elimination of *Trichomonas foetus* in bulls has thus far been developed.

CONTROL OF TRICHOMONIASIS IN HERDS

The following recommendations for the control of trichomoniasis in an infected herd are based on the data obtained in the present investi-

⁷ GARLICK, GEORGE G. TRANSMISSION OF BOVINE VENEREAL TRICHOMONIASIS THROUGH ARTIFICIAL INSEMINATION. VET. MED. 34 (1): 42-44. 1939.

gation: (1) Breed infected bulls only to infected cows or to those that have recovered from the infection; (2) breed clean bulls to heifers and to cows known to be free from infection; (3) breed cows with unknown histories artificially with semen from noninfected bulls.

SUMMARY

Fifteen cases of experimentally transmitted trichomoniasis are described in heifers and cows. In 10 of the cases there had not been any previous infection, and in 5 cases there was previous infection. In general, the cases of first infection were severe and those of second infection were mild. One case is described in a bull.

In one heifer transmission of *Trichomonas foetus* during pregnancy resulted in no ill effects. Another infected heifer did not show indications of oestrus. In seven cases infection of the females resulted in failure of early conception and in irregularity or temporary absence of oestrus. The infection was finally overcome and conception and normal gestation followed. In one heifer conception was followed by pyometra. One cow that had a vaginal infection as a heifer showed slight resistance to reinfection. In four cows, reinfection followed reexposure by coitus with an infected bull but without evident ill effects.

By intravaginal inoculation the incubation period was as short as 2 days; by coitus the incubation period averaged about 11 days. The data do not indicate that the method of transmission had any influence on the course of the disease.

Examinations of material from the vaginas of infected females revealed *Trichomonas foetus* in 58 percent of the tests.

Trichomoniasis has persisted for 20 months in a bull infected during coitus with an infected heifer.

Transmission of *Trichomonas foetus* occurred in every female served by an infected bull.

Infection of a bull with *Trichomonas foetus* has had no apparent effect on the animal's potency.

RELATION OF GAIN IN WEIGHT TO GAIN IN ENERGY CONTENT OF GROWING CHICKS¹

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INTRODUCTION

The gain in weight, or the quantity of feed required to make a definite amount of gain, is frequently used to compare the values of two or more rations or to judge the efficiency of one feed as compared with that of another. The literature on this subject is voluminous, and need not be reviewed in detail here. The gain in live weight has been used for comparing the efficiency of feeds or rations for growing chicks (8),² for fattening calves (5), lambs (6), and other animals (7), and for comparing the efficiency of proteins (2) or the deficiency of amino acids in proteins. Armsby (1) has pointed out that the energy content of equal weights of animal at different periods of fattening may be different, and Fraps (3) has shown that the productive energy required per pound of gain in weight is different in different feeding experiments.

Data collected in connection with a study of the utilization of the energy of feeds by chickens³ have shown that the relation of gain in live weight to feed eaten is not always a reliable measure of the relative energy values of feeds. Some of these data are presented and discussed.

PROCEDURE

The work was done with White Leghorn baby chicks, fed individually. The chicks (2 or 3 days old) were all fed a corn-meal ration for a preliminary period of about 7 days, and then divided into six groups. One group was killed and analyzed for protein and fat. Four groups, having equal average weights, were fed individually in battery brooders on the four rations that were to be compared. The sixth group was used for digestion experiments on the rations to be compared. The chicks were weighed weekly. At the end of the period, usually 21 days, the chicks were killed, the intestinal contents removed, paper pulp or some preservative was added, and an analysis made for protein and fat. The energy content of the chicks was estimated in Calories by the use of the factors 5.66 for fat and 9.35 for protein (4).

A corn-meal ration was used as the standard for comparison in experiments 6, 8, and 9. It consisted of corn meal, 56 percent; alfalfa-leaf meal, 6 percent; casein, 12 percent; yeast, 2 percent; wheat gray shorts, 20 percent; calcium carbonate, 1 percent; tricalcium phosphate, 1 percent; salt, 1 percent; and fish oil, 1 percent. For experiments 11, 12, 13, and 17 the standard ration was the same except that 0.2 percent of fortified fish oil was used instead of 1 percent as in the previous ration, and 56.8 percent of corn meal instead of 56 percent.

¹ Received for publication June 12, 1939. Technical contribution No. 529 of the Texas Agricultural Experiment Station.

² Italic numbers in parentheses refer to Literature Cited, p. 781.

³ FRAPS, G. S., and CARLYLE, E. C. UTILIZATION OF ENERGY OF WHEAT PRODUCTS BY CHICKENS. *Jour. Nutrition*, 1939, 18: In press.

In experiments 6, 8, and 9, 50 percent of corn meal was replaced by 50 percent of patent flour, or low-grade flour, or wheat bran, or wheat brown shorts, for the other rations. In experiments 11 and 12, 50.8 percent of corn meal was replaced by 50.8 percent of starch in one ration, 38 percent of corn meal was replaced by 38 percent of casein in a second ration, and 15 percent of corn meal was replaced by 15 percent of Wesson oil in a third ration. In experiment 13 corn meal was replaced by three different lots of corn bran. In experiment 17, 50 percent of corn meal was replaced by oat hulls or two kinds of kafir.

DATA AND DISCUSSION

Table 1 shows the average gains in live weight of the six chicks in each group, the percentages of protein and fat, the Calories per 100 grams, the gain in energy of the chicks, and the quantities of feed eaten. The data show that the differences in the rations may cause wide differences in the fat content of the chicks. In experiment 6 the chicks fed on the wheat-bran ration contained only 2.96 percent of fat, while those on the corn-meal ration contained 8.81 percent of fat, although the chicks ate considerably more of the wheat-bran ration than of the corn-meal ration. In experiment 8 the fat content was 3.70 percent in the chicks fed the wheat-brown-shorts ration and 8.04 percent for those on the corn-meal ration. Similar differences in the fat content are observed in the other experiments. The highest average fat content (12 percent) was found in experiment 12 from a ration in which 15 percent of corn meal was replaced by 15 percent of Wesson oil. The lowest average fat content (2.02 percent) was found in chicks fed a wheat-bran ration. These differences in fat content mean corresponding differences in energy content. The differences in energy content are not so great as those in fat content, however, since a large portion of the energy comes from the protein and the variations in protein content are both small and in the opposite direction to the variations in fat. That is, the protein slightly increases as the fat decreases. The energy content per 100 gm. is given in table 1, and the differences are appreciable. In experiment 6 the calories range from 196 per 100 gm. for chickens on the corn-meal ration to 146 calories per 100 gm. for those on the wheat-bran ration. The lowest is 144 calories in experiment 17 and the highest is 225 per 100 gm. in experiment 12. Thus, 1 gm. of live weight from chickens fed on one ration may represent much less energy than 1 gm. from chickens fed on another ration.

The grams of feed required to produce 1 gm. of gain and also 1 calorie of gain are given in table 2. The relative values are brought out more clearly when the rations are compared with the corn-meal ration as a standard equal to 100.

The relative quantity of feed required per calories of gain is greater than that required per gram of live weight in all the tests except those with the starch ration and the Wesson-oil ration in experiments 11 and 12. That is to say, the differences in most of the rations are greater than are indicated by the gains in live weight. The differences are especially large with the wheat-bran ration (experiments 6 and 9), the wheat-brown-shorts ration (experiment 8), the oat-hull ration (experiment 17), the corn-bran ration (experiment 13), and the casein ration (experiment 11). The quantity of feed required per gram of

gain in weight and that required per calorie of gain are parallel in most of the experiments, but there are some notable exceptions. Fed on the two Wesson-oil rations, in which 15 percent of corn meal was replaced by 15 percent of Wesson oil (experiments 11 and 12), the chicks required 11 and 13 percent more of the Wesson-oil ration than of the corn-meal ration to make the same gain in live weight, which is contrary to what would reasonably be expected from a ration with a higher feeding value. But for equal calories of gain, the Wesson-oil ration consumed was 95 or 84 percent of the corn-meal ration consumed. The chicks fed the Wesson-oil ration ate less and made less growth than those fed the corn-meal ration, but they stored much greater percentages of fat. The chicks fed the casein ration in experiment 12 required practically the same quantity of feed as those fed the corn-meal ration, in order to make the same gain in live weight, but they contained less fat and required 47 percent more feed to make the same gain in calories.

TABLE 1.—Average live weight, composition, and gain of energy of chicks and feed eaten

Experiment No.	Ration	Live weight		Composition		Energy per 100 grams of empty weight	Gain of energy	Feed eaten
		Beginning	Gain	Protein	Fat			
		Grams	Grams	Percent	Percent	Calories	Calories	Grams
6	Corn meal.....	65.9	125.8	20.09	8.81	196	264.4	275.2
	Patent flour.....	65.6	122.0	20.14	7.75	186	223.6	271.9
	Low-grade flour.....	65.9	115.7	20.34	6.15	173	209.1	277.2
	Wheat bran.....	65.8	111.4	20.84	2.96	146	151.8	356.5
8	Corn meal.....	60.5	149.2	20.34	8.04	190	289.2	288.6
	Patent flour.....	59.6	135.1	20.93	7.04	184	255.0	299.8
	Low-grade flour.....	59.9	141.1	20.79	5.04	165	224.9	289.2
	Wheat brown shorts.....	60.4	139.5	21.03	3.70	154	200.4	376.1
9	Corn meal.....	59.4	157.1	21.45	6.66	184	291.3	322.8
	Corn bran.....	60.2	155.0	23.04	3.13	160	236.6	442.2
	Wheat brown shorts.....	60.6	148.5	22.94	2.97	158	226.5	362.2
	Wheat bran.....	60.4	132.6	22.69	2.02	147	178.0	423.3
11	Corn meal.....	62.5	150.1	20.97	7.38	188	286.1	315.7
	Casein.....	62.9	94.0	21.62	2.62	147	120.3	220.0
	Starch.....	63.3	98.0	19.96	9.58	203	217.3	270.1
	Wesson oil.....	63.0	99.9	20.25	9.87	207	227.0	237.0
12	Corn meal.....	60.0	130.5	21.04	7.55	190	251.3	280.0
	Casein.....	60.6	102.2	21.39	2.84	148	129.9	213.1
	Starch.....	60.1	77.7	19.59	9.31	198	166.5	238.7
	Wesson oil.....	60.0	91.6	19.97	12.00	225	231.9	217.4
13	Corn meal.....	53.1	132.1	19.98	8.14	189	258.2	275.4
	Corn bran.....	52.7	137.0	21.33	3.78	156	207.0	363.8
	do.....	52.5	141.1	21.39	4.89	167	230.8	346.1
	do.....	52.9	130.5	21.38	4.16	160	197.6	415.1
17	Corn meal.....	52.3	124.4	20.59	8.41	195	264.3	275.7
	Oat hulls.....	52.9	111.7	21.49	2.36	144	154.7	392.9
	Blackhull kafir.....	53.0	130.2	20.54	8.26	193	269.0	290.0
	"Waxy endosperm" kafir.....	54.1	121.1	20.72	7.46	187	245.0	275.3

It is evident from the above discussion that gains in live weight or feed required per unit gain in live weight may not be correct measures of the energy values of feeds used in feeding experiments, since the energy contained in the gains may be different with the different groups of animals. The animals may contain different percentages of fat. The quantity of feed required per gram of gain would then not be in the same proportion as the quantity required per calorie of gain. Rations which appear to have the same values when gains in live weight are compared may have different values when gains in energy are compared.

TABLE 2.—*Feed required per gram of gain in live weight or calories*

Experiment No.	Ration	Feed required per gram of gain	Feed required per calorie of gain	Relative quantity required for gain in—	
				Weight	Calories
		<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>
6	Corn meal.....	2.19	1.04	100	100
	Patent flour.....	2.23	1.22	102	117
	Low-grade flour.....	2.40	1.33	110	127
	Wheat bran.....	3.20	2.35	146	226
8	Corn meal.....	1.93	1.00	100	100
	Patent flour.....	2.22	1.18	115	118
	Low-grade flour.....	2.05	1.29	106	129
	Wheat brown shorts.....	2.70	1.88	139	188
9	Corn meal.....	2.06	1.11	100	100
	Corn bran.....	2.85	1.87	139	169
	Wheat brown shorts.....	2.44	1.60	119	144
	Wheat bran.....	3.19	2.38	155	215
11	Corn meal.....	2.11	1.10	100	100
	Casein.....	2.34	1.83	111	166
	Starch.....	2.76	1.24	131	113
	Wesson oil.....	2.37	1.04	113	95
12	Corn meal.....	2.15	1.11	100	100
	Casein.....	2.09	1.64	97	147
	Starch.....	3.07	1.43	143	129
	Wesson oil.....	2.37	.94	111	84
13	Corn meal.....	2.09	1.07	100	100
	Corn bran.....	2.66	1.76	127	165
	do.....	2.45	1.50	118	141
	do.....	3.18	2.10	153	197
17	Corn meal.....	2.22	1.04	100	100
	Oat hulls.....	3.52	2.54	159	244
	Blackhull kafir.....	2.23	1.08	100	103
	"Waxy endosperm" kafir.....	2.27	1.12	102	108

The comparison of gains in live weight is convenient and practical, and no better method may be available for some purposes, especially for practical comparisons in feeding experiments. The possible inaccuracies here pointed out, however, should not be disregarded. In scientific work, the differences in energy content should be taken into consideration. Feed per calorie of gain is also not an exact method of comparison, since the feed used for maintenance is not considered. The maintenance requirements are allowed for when the productive energy is estimated (4, 5).

SUMMARY

The fat and energy content of chicks fed rations in which the feed tested replaced corn meal, were determined in six series of experiments. The fat content of the chicks ranged from a minimum of 2.02 percent to a maximum of 12.02 percent and the energy content from 144 to 225 calories per 100 gm. The energy content per gram of live weight was different for the different rations. The relative quantity of feed required per gram of gain in live weight was different from the relative quantity required per calorie of gain. In one experiment the chicks on a casein ration required practically the same quantity of feed as those on a corn-meal ration per unit of gain in live weight, but they required 47 percent more per calorie of gain. Chicks fed a ration in which 15 percent of Wesson oil replaced 15 percent of corn meal required in two experiments 11 or 13 percent more feed to produce the same gain in live weight as the corn-meal ration, although the Wesson-oil ration should have a higher productive energy. They required 5 or 16 percent less of the Wesson-oil ration to produce the same calories of gain, which is in accord with the higher productive

energy. Feed required per unit of gain in live weight is not a safe standard for comparing the feeding values of rations, though it may be a good practical one, especially for animals sold on a weight basis. In scientific work, the fact that equal gains in live weight do not necessarily mean equal gains in energy should not be overlooked.

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CORRELATED INHERITANCE IN OATS OF REACTION TO SMUTS, CROWN RUST, STEM RUST, AND OTHER CHARACTERS¹

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INTRODUCTION

Loose and covered smut, and stem and crown rust are among the most destructive diseases of oats (*Avena* spp.). These diseases, caused by the pathogens *Ustilago avenae* (Pers.) Jens., *U. levis* (Kell. and Sw.) Magn., *Puccinia graminis avenae* Erikss. and Henn., and *P. coronata avenae* Corda, respectively, result in heavy annual losses both in yield and quality of grain. Frequently heavy losses are incurred from the rusts, which occur in epidemic form over large areas.

The inheritance of reaction to the smuts and rusts of oats and its relation to other characters have been studied by a number of investigators. Such information is of great value to the plant breeder in developing strains of oats highly resistant to the major diseases and possessing desirable agronomic characters. The present study reports the results of experiments on the mode of inheritance of stem and crown rust and smut reaction in several oat crosses. In the cross Iowa No. 444 × Bond the mode of inheritance of certain kernel characters was investigated as well as the possible relation of these characters to smut and rust reaction.

MATERIALS

The progenies used in these studies consisted of the F₂ and F₃ generations of the crosses Iowa No. 444 (C. I. 2331) × Bond (C. I. 2733), Victoria (C. I. 2401) × Richland (C. I. 787), Carleton (C. I. 2378) × (Victoria-Richland, selection from XS1098), and (Victoria × Richland Sel. No. 5544-3) × State Pride (C. I. 1154), and the F₂ generations of Bond (C. I. 2733) × S. D. 334 (C. I. 2884), Nidar (C. I. 3318) × (Victoria × Richland, Sel. No. 5544-3), Anthony (C. I. 2143) × Victoria (C. I. 2401), and Bond (C. I. 2733) × Hawkeye (C. I. 2464). The F₁ generations of the first three crosses mentioned were obtained from T. R. Stanton, of the United States Department of Agriculture. The remainder of the crosses were made at the Wisconsin Agricultural Experiment Station.

All the parental varieties used belong to the species *Avena sativa* L. with the exception of Bond which belongs to *A. byzantina* C. Koch.

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The basis for the classification of Bond is given by Stanton and Murphy (27)³. Table 1 shows where the parental varieties were developed, their origin, and more important characters.

TABLE 1.—*Origin and description of parental varieties*¹

Variety	C. I. ² No.	Developed or introduced by—	Origin	Superior characters
Iowa No. 444..	2331	Iowa Agricultural Experiment Station.	Pure line selection from "Rustless" from Iowa.	High yield, moderate resistance to stem rust.
Bond.....	2733	U. S. Department of Agriculture; from Australia.	Selection from <i>Avena sterilis</i> × Golden Rain.	High resistance to crown rust and smut, stiff straw.
Victoria.....	2401	U. S. Department of Agriculture; from Uruguay.	Mass population of pure lines selected from native oats of Uruguay.	High resistance to crown rust and smut.
Richland.....	787	Iowa Agricultural Experiment Station.	Pure line selection from Kherson.	Early maturity, high resistance to stem rust.
Carleton.....	2378	U. S. Department of Agriculture.	Selection from Sixty-Day (Selection 165-1) × Markton.	Early, high resistance to smut, high yield.
Victoria × Richland.		do.....	Selection from Victoria × Richland (XS1098).	High resistance to stem rust and smut.
Do.....	{Sel. No. 5544-3}	do.....	do.....	High resistance to stem rust, crown rust, and smut.
State Pride....	1154	Wisconsin Agricultural Experiment Station.	Pure line selection from Kherson.	High yield, thin hull, early maturity.
S. D. 334.....	2884	South Dakota Agricultural Experiment Station.	Selection from (Markton × Richland) × (Swedish Select × Kilby Hullless).	High resistance to stem rust and smut.
Nidar.....	3318	University of Alberta; from Agricultural College at Aas, Norway.		Early maturity.
Anthony.....	2143	Minnesota Agricultural Experiment Station.	Selection from White Tartar (White Russian × Victory).	High yield, high resistance to stem rust.
Hawkeye.....	2464	Iowa Agricultural Experiment Station.	Selection from Richland × Green Russian.	High resistance to stem rust.

¹ Most of the data in this table were taken from the 1936 Yearbook of Agriculture (26).

² C. I. = Accession number of the Division of Cereal Crops and Diseases.

EXPERIMENTAL METHODS

FIELD STUDIES

The F₂ and F₃ generations of Iowa No. 444 × Bond, Victoria × Richland, and Carleton × (Victoria × Richland) were grown in 1936 and 1937, respectively, at the University Hill farms, Madison. The F₂ generations of the remaining crosses were grown in 1937. The F₂ and F₃ lines were planted in 5-foot rows with approximately 25 seeds per row. Parental varieties and State Pride were sown at intervals of approximately 30 rows. In 1937 State Pride was included to provide a susceptible host for the increase of crown and stem rust. Plants were classified as either resistant or susceptible to stem and crown rust.

GREENHOUSE STUDIES AND SMUT AND RUST REACTION

The smut studies were conducted in the greenhouse during the winters of 1936-37 and 1937-38. A composite inoculum of loose and covered smut, with a predominance of the former, was used for all tests. The hulls were removed from the kernels prior to inoculation. Approximately 25 kernels were sown for each F₃ line tested. The temperature of the greenhouse during the period from planting to emergence was kept at 20° C., but after emergence the temperature was lowered to 15°. The smut reaction was expressed as the percent-

³ Italic numbers in parentheses refer to Literature Cited, p. 802.

age of smutted plants. Check rows of State Pride were included in all tests.

The inoculum used in the crown and stem rust greenhouse studies, except where mentioned otherwise, was collected from the field and consisted of several physiologic races. The seedlings were grown in flats. The first leaf was inoculated with crown rust and later the second leaf with stem rust, by using the brushing method. The flats containing the inoculated plants were placed in a lighted moisture chamber for 24 hours at a temperature of 20° C., after which they were removed to a greenhouse kept at 18° C. A heavy epidemic of rust was secured in all cases, except where noted. The seedlings were classified according to their rust reaction into the following standard classes: 0, 1, 2, 3, and 4 described by Murphy (17) for *Puccinia*

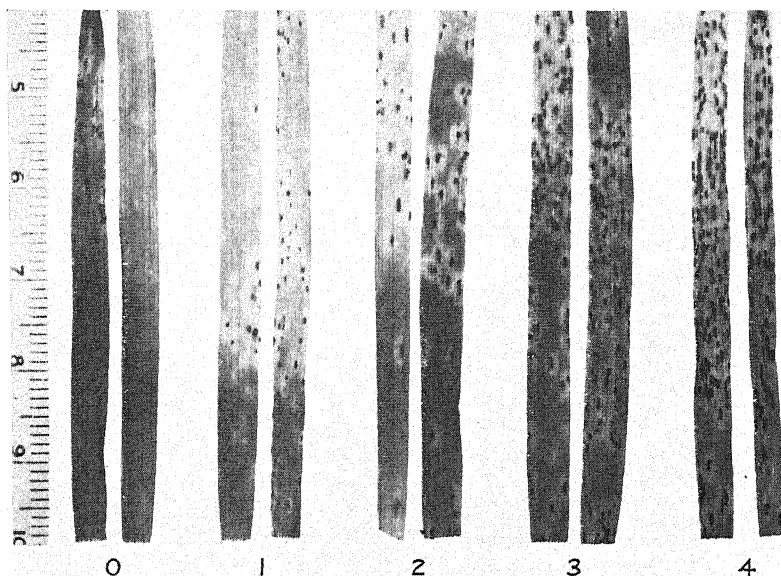


FIGURE 1.—Types of uredinial infection produced by *Puccinia coronata avenae* on seedling plants of oats: Type 0, no infection or necrotic flecks; type 1, small uredia surrounded by necrotic areas; type 2, uredia small to midsized in necrotic or chlorotic areas; type 3, uredia midsized in chlorotic areas, necrosis absent; type 4, large confluent uredia, no chlorosis or necrosis surrounding the uredia. Plants grown at 18° C.

coronata avenae, and by Gordon (12) and Levine and Smith (16) for *P. graminis avenae*.

The types of uredinial infection produced by *Puccinia coronata avenae* on seedling plants of oats are shown in figure 1. A large majority of the plants fell into classes 0, 1, and 4. For genetic analysis of the data, reaction types 0, 1, and 2 were considered resistant, and 3 and 4 susceptible (16). Representative plants of the different reaction classes were transplanted into pots and at heading were inoculated with both crown and stem rust.

During the winter of 1937–38, 67 F₃ lines of Iowa No. 444 × Bond (population 173) were tested in the seedling stage to each of the physiologic races 1, 7, and 46 of *Puccinia coronata avenae*.

EXPERIMENTAL RESULTS

INHERITANCE OF SMUT REACTION

The literature on the inheritance of smut reaction has been reviewed recently by Johnson (15) and by Austin and Robertson (2). The number of factor pairs involved in resistance to the smuts was found to vary considerably for the different crosses and smut collections used by the different investigators.

The inheritance of smut reaction was studied in the F_2 and F_3 generations of Iowa No. 444 \times Bond, and in the F_3 generation of Victoria \times Richland and (Victoria \times Richland Sel. No. 5544-3) \times State Pride. A heavy epidemic of smut was obtained for all crosses save that of Victoria \times Richland. The distribution of the F_3 and parental lines for percent of smutted plants, in 10-percent classes, is given in table 2, while the F_2 segregation is given in table 3.

TABLE 2.—Distribution of F_3 and parental lines for smut reaction to composite inoculum, of the crosses Iowa No. 444 \times Bond, Victoria \times Richland, and (Victoria \times Richland Sel. No. 5544-3) \times State Pride

Cross or parent	Lines in percent smut class indicated											Total lines	Average percent of smutted plants
	0	5	15	25	35	45	55	65	75	85	95		
Iowa No. 444 \times Bond (population 173)	No. 9	No. 20	No. 21	No. 32	No. 25	No. 11	No. 15	No. 6	No. 7	No. 4	No. 9	No. 159	Percent 35
Bond.....	1	4	1									6	5
Iowa No. 444.....										2	4	6	83
State Pride.....											7	7	99
Iowa No. 444 \times Bond (population 183)	15	12	18	13	6	8	1	5	7	2	4	91	30
Bond.....	5											5	0
Iowa No. 444.....									1	2	1	4	88
State Pride.....										1	4	5	94
Victoria \times Richland.....	23	13	8	7	4	3	2	1	1	2	1	65	19
Victoria.....	4											4	0
Richland.....						1	2	1				4	32
State Pride.....								4				4	63
5544-3 \times State Pride.....	28	12	17	19	10	3	5	3	1	4	10	112	28
5544-3.....	5											5	0
State Pride.....											5	5	99

TABLE 3.— F_2 smut reaction to composite inoculum for three populations of Iowa No. 444 \times Bond

Cross	Smut free		Smutted	
	Number		Number	Percent
Iowa No. 444 \times Bond.....	74	47	39	
	71	35	33	
	68	53	44	
Total.....	213	135	39	

The segregation for smut reaction was very similar for all crosses studied. The data agree closely with those found by Johnson (15) in the cross Black Mesdag \times Victory. Johnson assumed that there were two factor pairs governing the resistance of Black Mesdag: R a dominant factor for high resistance, or immunity; and P , a supplementary factor for partial resistance. According to this hypothesis one-fourth of the F_3 lines would be expected to possess the factor pair RR and have the resistance of the resistant parent, and one-sixteenth

of the F_3 lines would have the factorial constitution $rrpp$ and be as susceptible as the susceptible parent.

Two populations of Iowa No. 444 \times Bond were tested in the F_3 for smut reaction during the winter of 1936-37. The almost complete susceptibility of State Pride (99 percent smut) and the high percent of smutted plants obtained for Iowa No. 444 (93 percent) indicates that very few susceptible plants escaped smut in the test of Iowa No. 444 \times Bond (population 173). The percentage of smutted plants obtained for population 183, while not so high as that for population 173, was sufficient to give a good differentiation for smut reaction. Since the agreement between the two populations for smut reaction is essentially similar, a detailed discussion will be given only for population 173.

The most susceptible parental row of Bond, tested with population 173, contained 14 percent of smutted plants. It is logical to assume that all the F_3 lines of population 173 in the first two infection classes 0 and 5, and approximately one-half of those in the third class, 15, are as resistant as Bond. On this assumption approximately 40, or one-fourth of the F_3 lines, possess the resistance of Bond. Similarly, all the F_3 lines in the 95-percent infection class and most of those in the 85-percent infection class are considered as susceptible as Iowa No. 444. These make up 11 to 13 lines or approximately one-sixteenth of the total population. The number of F_3 lines obtained in the resistant and susceptible classes agree closely with that expected on the basis of two-factor pairs. On the basis of the F_3 segregation, where more than one-factor pair is involved, it is hardly possible to determine the genetic constitution of the F_3 lines with a smut reaction of different degrees of intermediacy between that of the parents.

Three populations of Iowa No. 444 \times Bond were tested for smut reaction in the F_2 generation during the winter of 1937-38. The data given in table 3 show that there was an average of 39 percent of smutted plants. No definite conclusions can be drawn from the F_2 data in regard to the number of factor pairs involved in determining smut reaction.

The F_3 generation of Victoria \times Richland was tested for smut reaction during the winter of 1936-37. The low percent of smutted plants obtained makes the interpretation of the data difficult. When a level of infection approaching maximum is not obtained, difficulty may be experienced in the genetic interpretation of the data, for plants which escape being smutted are classified as resistant. The average percent of smutted plants for State Pride was only 63, as compared with 94 and 99 obtained with Iowa No. 444 \times Bond. It is to be expected that a number of moderately resistant lines would be classified as being as resistant as Victoria on account of susceptible plants that escape infection. If this is taken into account, the 23 lines obtained with the resistance of Victoria agrees fairly closely with the 16 expected, on the hypothesis suggested previously. On the same hypothesis, one-sixteenth, or 4 F_3 lines, would be expected to be as susceptible as Richland. The data in table 2 show that 5 or 6 lines are as susceptible as Richland, and 4 are more susceptible. The transgressive segregation of lines more susceptible than Richland suggests that Richland may have a factor pair for resistance or partial resistance not possessed by Victoria. The evidence for transgressive

segregation, however, is not sufficiently clear to warrant any definite conclusions.

The F_3 generation of (Victoria \times Richland Sel. No. 5544-3) \times State Pride was tested for smut reaction during the winter of 1937-38. The resistant parent, Sel. No. 5544-3, developed no smutted plants, but State Pride, the susceptible parent, was 99 percent smutted. Assuming that Sel. No. 5544-3 possesses a factor pair for high resistance or immunity epistatic to a second factor pair for partial resistance, one-fourth, or 28 F_3 lines, would be as resistant as Sel. No. 5544-3, and one-sixteenth, or 7 lines, as susceptible as State Pride. This agrees very closely with the 28 resistant and 10 completely susceptible lines actually obtained.

The F_3 distributions for smut reaction of the crosses, Markton \times Early Champion and Markton \times Ligowa, reported by Coffman et al. (4), and of certain of the crosses studied by Reed and Stanton (22) are very similar to the distributions found in the present study. Coffman et al. and Reed and Stanton classify all F_3 lines with over 50 percent of smutted plants as susceptible, on which basis their results indicate a single factor. In this study only F_3 lines for which the percent of smutted plants was approximately the same as that for the susceptible parent were classed as susceptible. This separation was based on the assumption that susceptible F_3 lines would not have a higher percentage of escapes than the susceptible parent.

INHERITANCE OF CROWN RUST REACTION

Smith (24) recently reviewed the literature on the inheritance of resistance to crown rust. Parker (20) concluded that multiple factors controlled crown rust reaction. Davies and Jones (5, 6) and Dietz and Murphy (8) report a segregation of 3 resistant to 1 susceptible. Dietz and Murphy (8) in the cross Sunrise (resistant) \times Fulghum (susceptible), however, obtained an F_2 segregation of 13 susceptible to 3 resistant, which they explain upon the action of 2 factors, one of which is an inhibitor for resistance.

The seedling reaction to a composite collection of crown rust was studied for the F_3 of Iowa No. 444 \times Bond (population 183) in the greenhouse during the winter of 1936-37, and for three F_2 populations and several F_3 families in the F_4 of Iowa No. 444 \times Bond (population 183) during the winter of 1937-38. The F_3 seedling reaction of Iowa No. 444 \times Bond (population 173) to physiologic races 1, 7, and 46 was determined during the winter of 1937-38. Data were obtained on the mature plant reaction of the F_3 of Iowa No. 444 \times Bond during the summer of 1937 under a natural epidemic of crown rust.

The segregation for crown rust reaction of Iowa No. 444 \times Bond and tests of goodness of fit are given in tables 4 and 5. The segregation found suggests the presence of two factors, *S*, a factor for crown rust resistance, and *I*, a factor which partly inhibits the expression of *S*. On this assumption the genotype of the parents would be Iowa No. 444 (susceptible) *IIss*, and Bond (resistant) *iiSS*. The expression of the inhibitor, when heterozygous, was found to be different for the seedling and mature plant. In the seedling stage the inhibitor was effective in masking the expression of the *S* factor for resistance only when in the homozygous dominant condition. In the mature plant stage under field conditions, however, the inhibitor also was effective, when

heterozygous, in masking the *Ss* genotype but is not potent enough to mask the *ss* genotype.

TABLE 4.—The inheritance of crown rust reaction for the F_2 of Iowa No. 444 \times Bond, in the seedling stage, and tests of goodness of fit

Values	Segregation of population in indicated infection classes											
	694-1							694-2				
	Resistant				Susceptible			Resistant				
	0	1	2	Total	3	4	Total	0	1	2	Total	
Observed.....	31	12	9	52	15	34	49	21	8	16	45	
Expected.....				56.81			44.39				48.93	
χ^293							.75	
Range of <i>P</i>				0.50-0.30							0.50-0.30	

Values	Segregation of population in indicated infection classes											
	694-2				694-3							
	Susceptible			Total	Resistant				Susceptible			
	2	4	Total		0	1	2	Total	3	4	Total	
Observed.....	11	31	42		26	22	23	71	18	34	52	
Expected.....			38.07					69.19			53.81	
χ^211				
Range of <i>P</i>								0.80-0.70				

The seedling and mature plant reaction expected is shown in table 6.

The crown rust reaction of the F_2 and F_3 generations and selected F_4 lines of Iowa No. 444 \times Bond in tables 4 and 5 in all instances showed good agreement with that expected by the hypothesis suggested. F_1 plants tested in the seedling stage in the greenhouse and in the mature-plant stage in the field had a 1 to 2, and a 3 type of reaction, respectively. Humphrey and Coffman (13) report that, under greenhouse conditions, resistance to crown rust was dominant or intermediate in adult F_1 plants from crosses of resistant and susceptible varieties. The segregation of three F_2 populations in the seedling stage (table 4) gave good fits to the 9 resistant to 7 susceptible ratio. In F_3 lines originating from double heterozygous F_2 plants (*IiSs*) the ratio was 9 resistant to 7 susceptible in the seedling stage, and 11 susceptible to 5 resistant in the mature-plant stage. In these lines, both in the greenhouse and in the field, certain plants were more or less intermediate in their rust reaction, which made the classification into resistant and susceptible types somewhat difficult.

In order to provide an additional check upon the segregation of lines grown from *IiSs* plants, the F_4 seedling reaction was determined for seven F_3 families, 183-5 to 183-91, inclusive, which segregated in the ratio of 9 resistant to 7 susceptible in the F_3 seedling tests. The χ^2 values given in table 5 show good fits for each of the seven F_3 families and for the total of all families to the 7:4:4:1 ratio. This

segregation, as expected by the factorial hypothesis suggested, is the same as secured for the F_3 seedling test. In this particular test, the rust epidemic was not so heavy as in previous tests, so an occasional escape was found. The escapes, however, were not sufficiently frequent to upset the results.

TABLE 5.—*The inheritance of crown rust reaction for the cross Iowa No. 444 × Bond and tests of goodness of fit*

Population and inoculum	Observed or expected	Seedling ¹ showing indicated reaction				χ values	Range of P
		Resistant	Susceptible	3R:1S ²	9R:7S ²		
		Number	Number				
F ₃ 173, race 1.....	Observed.....	4	31	12	20	2.08	0.70-0.50
F ₃ 173, race 7.....	Observed.....	3	30	16	18	.48	.95-.90
F ₃ 173, race 46.....	Observed.....	5	28	16	18	.35	.95
	Expected.....	4.19	29.33	16.76	16.76		
F ₃ 183, composite.....	Observed.....	4	40	24	21	.67	.90-.80
	Expected.....	5.56	38.94	22.25	22.25		
F ₄ 183-5, composite.....	Observed.....	1	8	2	4	1.15	.80-.70
	Expected.....	.94	6.57	3.75	3.75		
F ₄ 183-30, composite.....	Observed.....	0	4	4	4	1.71	.70-.50
	Expected.....	.75	5.25	3	3		
F ₄ 183-32, composite.....	Observed.....	1	4	2	9	8.54	.05-.02
	Expected.....	1	7	4	4		
F ₄ 183-49, composite.....	Observed.....	1	7	2	4	.85	.90-.80
	Expected.....	.88	6.13	3.5	3.5		
F ₄ 183-52, composite.....	Observed.....	2	3	6	3	4.88	.20-.10
	Expected.....	.88	6.13	3.5	3.5		
F ₄ 183-78, composite.....	Observed.....	1	5	5	2	1.55	.70-.50
	Expected.....	.81	5.69	3.25	3.25		
F ₄ 183-91, composite.....	Observed.....	1	6	0	2	3.70	.30-.20
	Expected.....	.56	3.94	2.25	2.25		
F ₄ Total composite.....	Observed.....	7	37	21	28	1.76	.70-.50
	Expected.....	5.81	40.69	23.25	25.25		
Population and inoculum	Observed or expected	Mature plant ³ , showing indicated reaction				χ values	Range of P
		Resistant	Susceptible	3R:1S	11S:5R		
		Number	Number				
F ₃ 173, composite.....	Observed.....	12	79	37	45	1.25	0.80-0.70
	Expected.....	10.81	75.67	43.24	43.24		
F ₃ 183, composite.....	Observed.....	7	79	38	45	2.15	.70-.50
	Expected.....	10.56	73.94	42.23	42.25		

¹ Grown in greenhouse.

² R=resistant, S=susceptible.

³ Grown in field.

Sixty-seven F_3 lines of Iowa No. 444 × Bond (population 173) were tested in the seedling stage for their reaction to each of physiologic races 1, 7, and 46 of *Puccinia coronata avenae*. The results (table 5) show that the seedling reaction to individual races of crown rust was the same as that obtained when a composite inoculum was used. The agreement between the reaction of the different lines for the three races was close. A few lines, particular in the two segregating classes, were reversed in their reaction. This may be due to the action of modifying factors.

A close relationship was found between the rust reaction of seedling and mature plants. This is in agreement with the results reported by Parker (20) and Smith (24).

TABLE 6.—Seedling and mature plant reactions expected in the cross Iowa No. 444 (*IISs*) × Bond (*iiSS*)

Generation genotype	Phenotypic expression in 1—				Generation genotype	Phenotypic expression in 1—			
	Seedling		Mature plant			Seedling		Mature plant	
	F ₁ or F ₂ plant	F ₃ line	F ₁ or F ₂ plant	F ₃ line		F ₁ or F ₂ plant	F ₃ line	F ₁ or F ₂ plant	F ₃ line
F ₁ , <i>IiSs</i>	<i>r</i>	-----	<i>s</i>		F ₂ —Cont.				
F ₂ :				<i>s</i>	4 <i>IiSs</i>	<i>r</i>	9 <i>r</i> :7 <i>s</i>	<i>s</i>	11 <i>s</i> :5 <i>r</i>
1 <i>IISS</i>	<i>s</i>	<i>s</i>	<i>s</i>		2 <i>Iiss</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>
2 <i>IISs</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	1 <i>iiSS</i>	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
1 <i>Iiss</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>	2 <i>iiSs</i>	<i>r</i>	3 <i>r</i> :1 <i>s</i>	<i>r</i>	3 <i>r</i> :1 <i>s</i>
2 <i>iiSS</i>	<i>r</i>	3 <i>r</i> :1 <i>s</i>	<i>r</i>	3 <i>r</i> :1 <i>s</i>	1 <i>iiss</i>	<i>s</i>	<i>s</i>	<i>s</i>	<i>s</i>

¹ *r*=resistant; *s*=susceptible.

INHERITANCE OF STEM RUST REACTION

The literature on the inheritance of stem rust reaction in oats has been reviewed by Smith (24). Most investigators, whether they have used a complete inoculum or individual races, have found that resistance to stem rust was dominant and inherited on a single-factor basis.

The seedling and mature plant reaction to a composite collection of stem rust was studied for the F₃ of Iowa No. 444 × Bond, Carleton × (Victoria × Richland), and Victoria × Richland in the greenhouse during the winter of 1936–37. A heavy natural epidemic of stem rust occurred in the oat-breeding nursery during the summer of 1937. Data were taken for the F₃ reaction of the above crosses and the F₂ reaction of several other crosses. The segregation for stem rust reaction and tests of goodness of fit for the F₃ data are given in table 7, and for the F₂ data in table 8.

F₁ plants of Iowa No. 444 × Bond both in the seedling stage in the greenhouse and in the mature plant stage in the field were resistant to stem rust. This is in agreement with the results reported by Humphrey and Coffman (13).

TABLE 7.—F₃ segregation for stem-rust reaction to composite inoculum for the crosses Iowa No. 444 × Bond, Carleton × (Victoria × Richland) and Victoria × Richland, and tests of goodness of fit

Crosses and place grown	Observed or expected	F ₃ lines that were—			χ ² values	Range of <i>P</i>
		Resistant	Segregating	Susceptible		
		Number	Number	Number		
Iowa No. 444 × Bond (173); field.....	{ O E	47 44.25	79 88.50	51 44.25	2.07	0.50–0.30
Iowa No. 444 × Bond (183); field.....	{ O E	47 42.25	79 84.50	43 42.25	.91	.70–.50
Iowa No. 444 × Bond (183); greenhouse.	{ O E	22 19.75	37 39.50	20 19.75	.42	.90–.80
Carleton × (Victoria × Richland); field.	{ O E	29 29	63 58	24 29	1.29	.70–.50
Carleton × (Victoria × Richland); greenhouse.	{ O E	21 21	48 42	15 21	2.57	.30–.20
Victoria × Richland; field.....	{ O E	12 20.25	44 40.50	25 20.25	4.78	.10–.05
Victoria × Richland; greenhouse.....	{ O E	4 10.5	26 21	12 10.5	5.43	.10–.05

TABLE 8.— F_2 segregation for stem-rust reaction of several crosses under conditions of natural infection in the field, and tests of goodness of fit

Cross	Observed or expected	F_2 plants—		χ^2 values	Range of P
		Re- sistant	Sus- ceptible		
		<i>Number</i>	<i>Number</i>		
Bond \times C. I. 2884.....	{ O	152	52	0.03	0.90-0.80
	{ E	153	51		
5544-3 \times State Pride.....	{ O	175	54	.25	.70- .50
	{ E	171.75	57.25		
Nidar \times 5544-3.....	{ O	52	22	.88	.50- .30
	{ E	55.5	18.5		
Anthony \times Victoria.....	{ O	73	34	2.62	.20- .10
	{ E	80.25	26.75		
Bond \times Hawkeye.....	{ O	100	44	2.37	.20- .10
	{ E	108	36		

The segregation of the F_3 lines for stem-rust reaction gave fair to good fits to the ratio of 1 resistant: 2 segregating: 1 susceptible, for three crosses studied, both in the seedling and mature-plant stage. Both of the P values for Victoria \times Richland lie between the 0.10 and 0.05 points, which, being on the border line of significance, is indicative that the observed segregation departs somewhat from the theoretical. These low P values are largely the result of too few resistant lines. The number of plants in the heterozygous lines segregating 3 resistant:1 susceptible in the field and greenhouse were 554:193, and 421:142, respectively. The P values lay between 0.70 and 0.50, and 0.95 and 0.90, respectively. These values suggest that the small number of F_3 resistant lines found is probably due to chance. The F_2 data for the five crosses given in table 8 all show good fits to the ratio of 3 resistant:1 susceptible.

A separation of the composite inoculum by means of a differential host series according to the key given by Stakman et al. (25) indicated the presence of physiologic races 2, 5, and 7. Welsh (28) and Smith (24) both report that reaction to physiologic races 1, 2, 3, 5, and 7 are dependent upon the same factor pair.

Considerable variation was found in both the number and size of the uredinal pustules within resistant and susceptible lines of all crosses in the field. Separation of homozygous and heterozygous resistant plants was not possible, based upon the number and size of rust pustules. This variation in the number and size of the uredinal pustules of both resistant and susceptible lines was due largely to differences in maturity of the lines. This is illustrated in figure 2. Both the late-resistant and the late-susceptible plants had a greater number and larger uredinal pustules than the early plants. This same relation held for the parental varieties, as the plants adjacent to the alley were a few days later in maturing than those located in the center of the rows. The rust lesions on the late-resistant plants, while numerous, were of a restricted type.

The relation between seedling and mature plant reaction was studied in the F_3 of the crosses Iowa No. 444 \times Bond, Victoria \times Richland, and Carleton \times (Victoria \times Richland). Representative plants, classified according to their seedling reaction as having 0, 1, 2, 3, or 4 type of pustules were transplanted into pots and shortly after heading were tested for mature plant reaction in the greenhouse. Also, the relation

was studied for F_3 lines between seedling reaction in the greenhouse and mature-plant reaction in the field. In both instances the agree-

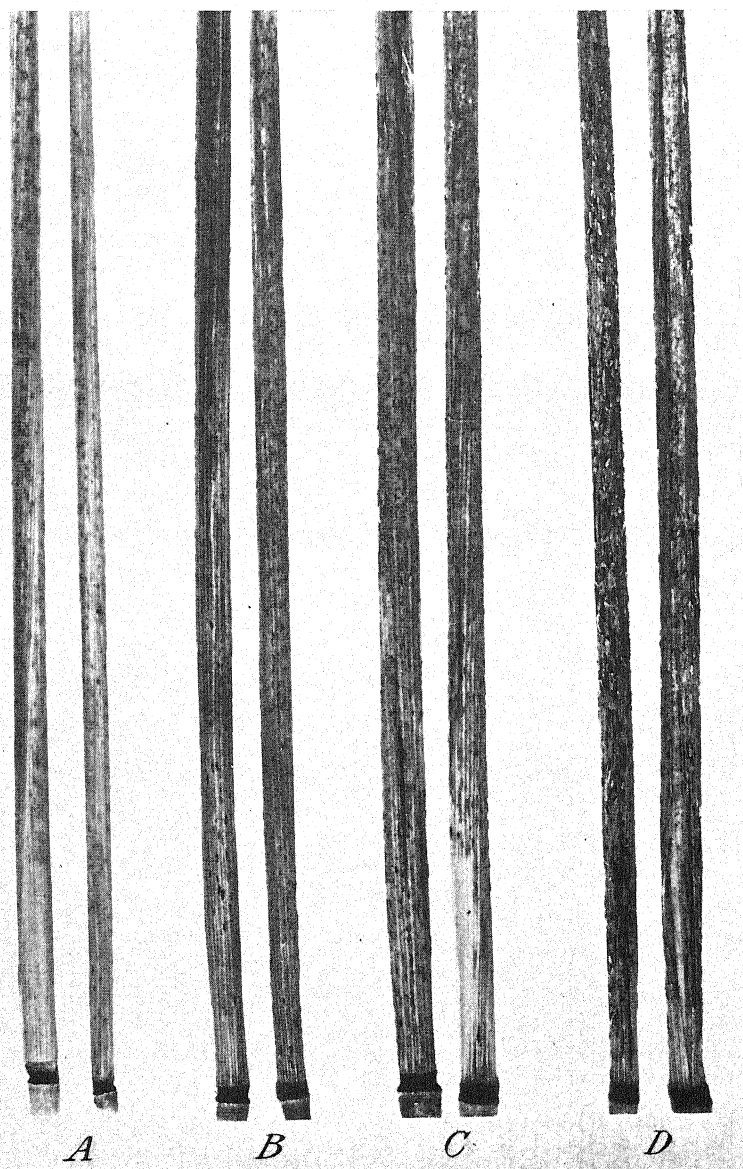


FIGURE 2.—Number and size of uredinial pustules, developing in the field, for F_3 segregates of Iowa No. 444 \times Bond: A, Early resistant; B, late resistant; C, early susceptible; D, late susceptible.

ment between seedling and mature-plant reaction was very close. This is in agreement with the results of other investigations, reviewed by Levine and Smith (16).

INHERITANCE OF KERNEL CHARACTERS FOR THE CROSS IOWA NO. 444 \times BOND

The inheritance of 7 kernel characters was studied in the F_1 , F_2 , and F_3 generations of Iowa No. 444 \times Bond. Iowa No. 444 belongs to *Avena sativa* and Bond to *A. byzantina*. Both species have 21 pairs of chromosomes. A description of the 2 parents for the characters studied, presented in summary form in table 9, is given below.

TABLE 9.—Comparison of Iowa No. 444 and Bond for several kernel characters

Variety	C. I. No.	Basal hairs		Basal articulation	Rachilla attachment	Lemma color	Awning	Ratio kernel width to length
		Length	Number					
Iowa No. 444.....	2331	Short....	Few....	Solid....	Primary	White....	Absent.....	Narrow.
Bond.....	2733	Medium long.	Many..	Sucker..	Secondary	Reddish yellow.	Weak awns present.	Wide.

Figure 3, which shows representative kernels of Iowa No. 444, F_1 of Iowa No. 444 \times Bond, and Bond, illustrates the main differences in kernel characters between these two varieties.



FIGURE 3.—Representative primary (a) and secondary (b) kernels, of Iowa No. 444 (A), primary kernel of F_1 Iowa No. 444 \times Bond (B), and primary (a) and secondary (b) kernels of Bond (C).

Bond separates from its pedicel by abscission, leaving a small distinct basal scar, commonly called sucker mouth, which is surrounded by a dense tuft of medium long hairs. The second floret separates from the first by basifracture, leaving the rachilla persistent to the second floret. The kernel of Bond is reddish yellow in color, wide in relation to its length, and bears a weak awn.

Iowa No. 444 separates from its pedicel by fracture leaving no scar at the base, commonly called solidified base, which is surrounded by

a few short hairs. The second floret separates from the first by disarticulation leaving the rachilla persistent to the first floret. The kernel of Iowa No. 444 is white, narrow, and awnless.

The F_1 kernels showed a partial dominance of the kernel characters of Iowa No. 444. Table 10 gives the modes of inheritance and tests of goodness of fit for the characters studied based on the segregation of the F_3 lines. A good fit of the observed to the expected ratio is shown for most of the characters. In several instances the P values lie between 0.98 and 0.95, as for example the yellow kernel color and awning for population 173, which indicates a closer agreement between the observed and expected ratios than expected by chance. The P value for basal hair length of population 173 lies between 0.05 and 0.02. This poor fit is accounted for by too many lines homozygous for short basal hairs and too few for long basal hairs. This same tendency, while not significant, is present in population 183. The number of plants segregating into 3 short and intermediate to 1 long basal hair for the segregating lines of populations 173 and 183 was 890:257, and 954:287, respectively. The P values were between 0.05 and 0.02, and 0.20 and 0.10, respectively.

TABLE 10.—Inheritance of several kernel characters in the F_3 lines of Iowa No. 444 \times Bond and tests of goodness of fit

Class and character	Population 173				Population 183			
	Observed	Expected	χ^2	Range of P	Observed	Expected	χ^2	Range of P
Basal hair length:	Number	Number			Number	Number		
Short.....	51	45	1.9	0.20-0.10	57	42.8	7.59	0.05-0.02
Segregation.....	91	90			82	85.6		
Long.....	38	45			32	42.8		
Type of basal articulation and basal hair number:								
Solid, few.....	46	45	.12	.95-.90	42	42.8	.04	.98-.95
Segregating.....	91	90			86	85.6		
Sucker, many.....	43	45			43	42.8		
Rachilla attachment:								
Primary.....	41	44.8	.71	.70-.50	46	42.8	.72	.70-.50
Segregating.....	89	89.6			80	85.6		
Secondary.....	49	44.8			45	42.8		
Red lemma color:								
Red.....	42	44.8	.22	.90-.60	37	42.8	2.44	.30-.20
Segregating.....	91	89.6			83	85.6		
Red absent.....	46	44.8			51	42.8		
Yellow lemma color:								
Yellow present.....	42	44.8	.23	.90-.80	42	42.8	.04	.98-.95
Segregating.....	92	89.6			86	85.6		
Yellow absent.....	45	44.8			43	42.8		
Awning:								
Awnless and partly awned.....	47	44.8	.15	.95-.90	43	42.3	.05	.98-.95
Segregating.....	88	89.6			83	84.6		
Awned.....	44	44.8			43	42.8		
Partial awning:								
Awnless.....	12	11.75	.007	.95-.90	18	10	8.53	<.01
Partly awned.....	35	35.25			22	30		
Ratio of kernel width to length:								
Wide.....	11	10.5	1.79	.80-.70	12	11.19	1.95	.90-.80
Intermediate.....	25	21.0			24	22.38		
3 intermediate: 1 wide.....	38	42.0			42	44.75		
3 intermediate: 1 narrow.....	45	42.0			50	44.75		
1 wide: 14 intermediate: 1 narrow.....	38	42.0			43	44.75		
Narrow.....	11	10.5			8	11.19		

The characters basal hair length and number, basal articulation, and rachilla attachment were monogenic in their inheritance. Fraser (10) for the cross Burt \times Sixty-Day, and Shaw and Bose (23) for crosses between *Avena sativa* and *A. sterilis*, found basal hair length

and basal articulation monogenic. Shaw and Bose (23) obtained a dihybrid ratio for basal hair number. Florell (9) reports that the type of floret separation in crosses between *A. sativa* and *A. sterilis* is conditioned by a unit factor.

Two-factor pairs were found to govern the inheritance of lemma color, awning, and ratio of kernel width to length. The reddish-yellow color of Bond is the result of the interaction of two-factor pairs, one pair for red, the other for yellow. The color of Bond apparently is similar to that reported for Burt by Fraser (10). The segregation of the F_3 lines gave a good fit to the ratio of 1 absent and partly awned; two segregating: one fully awned. Indications, however, were obtained which suggested the presence of a second pair of genes for awning.

The F_3 lines classified as awnless (all plants awnless) and partly awned (some plants awnless and others partly awned) segregated in a ratio of 1 awnless : 3 partly awned, for population 173. The segregation obtained for population 183, however, is a poor fit to the 3 : 1 ratio. The number of partly awned plants in the F_3 lines classified as partly awned varied in number from 1 to practically all of the plants in the lines. Also, the number of spikelets bearing awns on the primary kernels varied from 1 or 2 to practically all of the spikelets. These observations indicate that the expression of this second factor pair for awning is readily influenced by the environment or by other genetic factors. Eight of the approximately 1,500 F_3 plants examined in the fully awned F_3 lines were found to be fatuoids.

The segregation for the ratio of kernel width to length indicates the presence of two-factor pairs. Approximately one-sixteenth of the F_3 lines were homozygous for the wide-kernel type of Bond and one-sixteenth were homozygous for the narrow-kernel type of Iowa No. 444. F_3 lines homozygous for kernel width intermediate between that of the two parents composed approximately one-quarter of the total population.

TABLE 11.—Linkage intensities between the genes for several kernel characters in two populations of Iowa No. 444 \times Bond

Population and character ¹	Linkage intensity of character indicated				
	Yellow lemma color	Basal articulation and basal hair number	Awning	Rachilla attachment	Hair length
Population 173:					
Basal articulation and basal hair number	39.1 \pm 3.8				
Awning	39.9 \pm 3.8	0.7 \pm 0.4			
Rachilla attachment	40.0 \pm 3.8	17.3 \pm 2.3	17.3 \pm 2.3		
Hair length	53.4 \pm 5.2	50.0 \pm 5.0	46.0 \pm 4.8	33.9 \pm 3.5	
Red lemma color	53.4 \pm 5.2	51.5 \pm 5.1	46.9 \pm 4.8	31.8 \pm 3.4	30.0 \pm 0.8
Population 183:					
Basal articulation and basal hair number	29.5 \pm 3.5				
Awning	20.5 \pm 3.5	3.1 \pm .9			
Rachilla attachment	43.6 \pm 4.8	26.6 \pm 3.1	27.8 \pm 3.2		
Hair length	51.2 \pm 5.2	50.0 \pm 5.1	49.1 \pm 5.1	33.8 \pm 3.7	
Red lemma color	52.1 \pm 5.2	54.2 \pm 5.3	50.0 \pm 5.1	39.7 \pm 4.0	9.0 \pm 1.8

¹ Linear order of genes: Population 173: Yellow lemma color, 39.1; basal articulation and basal hair number, 0.7; awning, 17.3; rachilla attachment, 31.8; red lemma color, 3.0; hair length. Population 183: Yellow lemma color, 29.5; awning, 3.1; basal articulation and basal hair number, 26.6; rachilla attachment, 33.8; hair length, 9.9; red lemma color.

Table 11 shows the linkage intensities between the kernel characters which were calculated from the F_3 data by the method of maximum likelihood described by Immer (14). The linear order and distance between the genes as determined from two populations of Iowa No. 444 \times Bond are given in footnote 1, table 11. In figure 4 are shown kernels from F_3 plants of Iowa No. 444 \times Bond depicting cross-overs between several kernels characters. The linkage between the characters basal articulation and basal hair number was complete, indicating that the same gene pair or two closely linked pairs of genes governed the expression of these two characters. The linear order of the genes as determined for the two populations differed in two respects. The genes for awning, as determined from population 173,

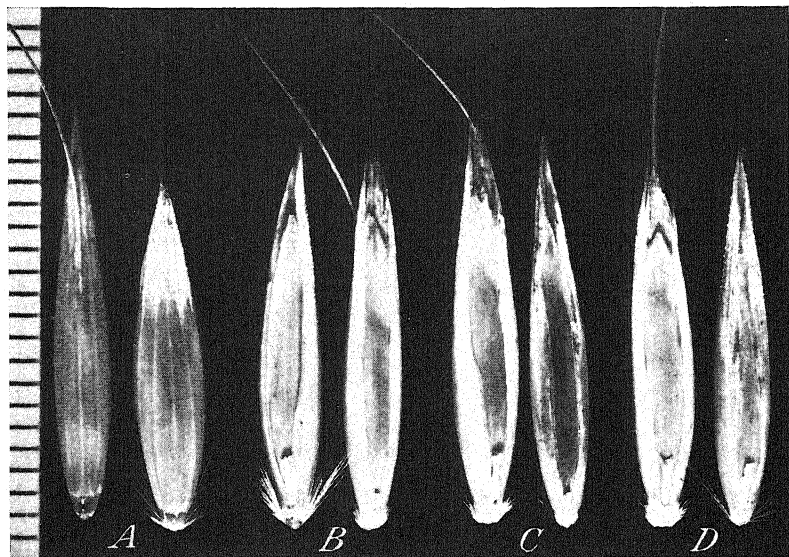


FIGURE 4.—Kernels from F_3 plants of Iowa No. 444 \times Bond showing cross-overs between kernel characters: A, Awning and basal articulation; B, basal hair length and rachilla attachment; C, rachilla attachment and basal articulation; D, basal hair length and basal articulation.

lay between those for basal articulation and rachilla attachment, whereas for population 183 the order of the genes for basal articulation and awning was reversed. The positions determined for the genes governing red lemma color and basal hair length were reversed in the two different populations. Further study would be necessary to determine the exact linear order of these genes.

The segregation of F_3 lines for width of kernel in relation to the other characters indicated that one of the factor pairs governing this character is closely linked with the factor for rachilla attachment.

Frazer (10) from a study of the cross Burt \times Sixty Day found a strong linkage between the factors for awning, Burt type of basal articulation, and medium basal hairs. Shaw and Bose (23), with interspecific crosses between *Avena sativa* and *A. sterilis* var. *Culta*, obtained crossing-over percentages of 17, 23, and 3.3 between the genes for weak awn and degree of basal pubescence. They, however, obtained no evidence of linkage between basal hair length and num-

ber. Smith (24) in the cross Gopher \times Rainbow found a linkage between lemma color, awning, and basal hairs.

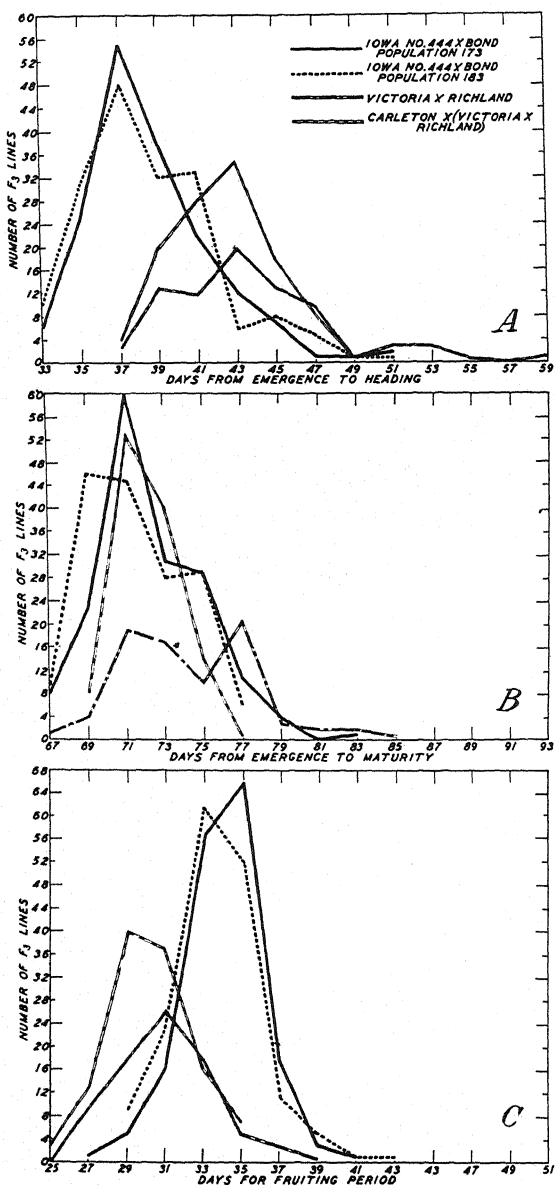


FIGURE 5.—Distribution of days from emergence to heading (A), to maturity (B), and for fruiting period (C) of F₃ lines for the crosses Iowa No. 444 \times Bond, Victoria \times Richland, and Carleton \times (Victoria \times Richland).

Nishiyama (18) from the study of a series of interspecific crosses, concludes that the *sativa* complex is partially dominant to both the *fatua* and *sterilis* complexes and the *sterilis* complex in turn partially

dominant to the *fatua* complex. Florell (9) reports that the complex of characters, oval pitted callus at the base of the spikelet, hairy ring around the callus, hairy rachilla, and strongly geniculate awns on the two lower florets is closely linked in *Avena sterilis* with the factor for the *sterilis* rachilla. Studies of the cross *A. sativa* × *A. fatua* which have been reviewed by Philp (21), and Aamodt, Johnson, and Manson (1), indicate a complete linkage between the characters of the fatuoid complex, basal articulation, pubescence of base and rachilla, awning, and base type of upper grain. The linkage relationships found in the cross Iowa No. 444 × Bond indicate that other kernel characters, besides those corresponding to the fatuoid complex, are located on the same chromosome.

INHERITANCE OF EARLINESS

The dates when the first, approximately 50 percent, and the last plant headed and matured were recorded for each F_3 and parental line of the crosses Iowa No. 444 × Bond, Victoria × Richland and Carleton × (Victoria × Richland selection from XS1098) in 1937. These data were used in calculating the number of days from emergence to heading and to maturity and length of fruiting period for each line. The fruiting period was taken as the number of days between heading and maturity. The distributions of these data are shown in figure 5, while in table 12 are given the means and standard deviations. The simple correlation coefficients between the three characters are given in table 13.

TABLE 12.—Means and standard deviations for days from emergence to heading and to maturity, and for fruiting period of F_3 and parental lines for the crosses Iowa No. 444 × Bond, Victoria × Richland, and Carleton × (Victoria × Richland)

Cross or parent	Number	Emergence to heading		Emergence to maturity		Fruiting period	
		Mean †	Standard deviation	Mean †	Standard deviation	Mean †	Standard deviation
		Days	Days	Days	Days	Days	Days
F_3 Iowa No. 444 × Bond (173).....	167	38.2±0.23	2.91	71.8±0.21	2.72	33.6±0.15	2.00
Iowa No. 444.....	8	44.0±.18	.53	72.6±.27	.75	28.8±.25	.71
Bond.....	8	37.8±.16	.45	73.9±.23	.64	36.1±.30	.84
F_3 Iowa No. 444 × Bond (183).....	163	37.9±.27	3.42	71.0±.20	2.55	33.2±.17	2.18
Iowa No. 444.....	10	44.2±.25	.79	73.2±.25	.79	28.9±.31	.99
Bond.....	10	37.2±.13	.41	73.8±.25	.79	36.3±.30	.96
F_3 Victoria × Richland.....	80	43.2±.36	3.18	73.9±.33	2.91	30.7±.28	2.50
Victoria.....	10	55.9±.28	.87	84.1±.29	.74	28.2±.25	.79
Richland.....	10	39.8±.20	.63	70.1±.31	.99	30.3±.46	1.46
F_3 Carleton × (Victoria × Richland).....	116	41.9±.23	2.47	71.6±.14	1.46	29.7±.19	2.02
Carleton.....	10	40.7±.34	1.06	71.3±.39	1.25	30.6±.31	.97
Victoria × Richland.....	10	43.3±.68	2.16	71.3±.42	1.33	27.9±.48	1.52

† Standard error of the mean shown.

The distributions, both for days from emergence to heading and to maturity, are skewed towards that of the earlier parent, which indicates a partial dominance of earliness. Bond headed approximately 6 days earlier than Iowa No. 444 and matured 1 day later, thus requiring about 7 days longer for fruiting period. The distribution of the F_3 lines for heading shows a preponderance of the lines as early as, or earlier than, Bond, but very few lines later than Iowa No. 444.

In regard to maturity, over one-half of the lines were earlier than either parent, and only a few were later. These distributions suggest that both parental varieties possess factors for earliness which are partly dominant over lateness. The distribution for length of fruiting period is massed towards Bond, the parent with the longer fruiting period.

TABLE 13.—Simple correlation coefficients between days from emergence to heading and to maturity and fruiting period of the crosses shown in table 12

Variables correlated	Iowa No. 44×Bond		Victoria×Richland	Carleton× (Victoria×Richland)
	Population 173	Population 183		
Heading to maturity.....	+0.68	+0.72	+0.70	+0.47
Heading to fruiting.....	-.60	-.47	-.55	-.81
Maturity to fruiting.....	+0.07	+0.26	+0.17	+0.10
1-percent level.....	±.21	±.21	±.29	±.24
Pairs.....number..	167	163	80	116

In the cross Victoria × Richland approximately one-quarter of the F_3 lines headed and matured as early as Richland, the early parent, and one-sixteenth as late as Victoria. The distribution suggests that Richland differs from Victoria by at least two-factor pairs for earliness. The (Victoria × Richland) parent used in the cross with Carleton is shown to be heterozygous for days from emergence to heading. Consequently, an interpretation of the F_3 data is not feasible.

The partial dominance of earliness is in agreement with the results reported by Noll (19), Garber and Quisenberry (11), and Shaw and Bose (23). It is very difficult to determine the number of factor pairs involved in the inheritance of a character like earliness. The data suggest, however, that possibly multiple factors are involved in the cross Iowa No. 444 × Bond and at least two in the cross Victoria × Richland. The factorial explanation offered by other investigators varied with the crosses studied. Noll (19) reports multiple factors, Caporn (3) three factors, and Garber and Quisenberry (11) two factors, to govern earliness. Shaw and Bose (23) and De Villiers (7) obtained evidence of one factor in certain crosses and multiple factors in others.

The correlations between heading and maturity were significant in all cases. The significant negative correlations between heading and fruiting show that in general lines which headed earlier had a longer fruiting period than those which headed later. This was particularly striking in the cross Victoria × Richland.

RELATION BETWEEN CHARACTERS

The independence or association of smut, crown and stem rust reaction with each other and with the characters of days from emergence to heading and to maturity, basal articulation, and basal hair length was measured by χ^2 for Iowa No. 444 × Bond. Since all the kernel characters studied were linked, basal articulation and basal hair length, between which the crossing-over was approximately 50 percent, were used in the χ^2 determinations. The results presented in table 14 show, with two exceptions, that the characters compared are inherited independently. These exceptions, for which the P value

lies between 0.05 and 0.02, are crown rust reaction in the field and heading for population 173, and heading and basal hair length for population 183. The P values for a comparison of the same characters of populations 183 and 173 lie between 0.80 and 0.70, and 0.20 and 0.10, respectively. Since at least 2 χ^2 values out of the 49 tested should exceed the 0.05 level by chance and since the P values for the corresponding comparisons in the other population indicate independence, the characters in the two cases discussed above are more than likely inherited independently.

TABLE 14.— χ^2 tests for independence or association of smut, crown and stem rust reaction, and other characters in the cross Iowa 444 \times Bond

Characters	Population 173			Population 183		
	χ^2 values	Degrees of freedom	Range of P	χ^2 values	Degrees of freedom	Range of P
Smut and crown rust reaction ¹	5.54	6	0.50-0.30	5.37	6	0.50-0.30
Smut and stem rust reaction ¹	5.86	8	.70-.50	4.59	8	.90-.80
Smut reaction and heading	6.76	8	.70-.80	12.47	8	.20-.10
Smut and reaction and maturity	15.50	8	.10-.05	5.05	8	.80-.70
Smut reaction and basal articulation	4.50	8	.90-.80	4.24	8	.50-.30
Smut reaction and basal hair length	7.91	6	.30-.20	8.37	8	.50-.30
Crown and stem rust reaction ¹	1.72	6	.95-.90	4.25	6	.70-.50
Crown rust reaction ¹ and heading	14.68	6	.05-.02	3.19	6	.80-.70
Crown rust reaction and maturity	7.40	6	.30-.20	12.55	6	.10-.05
Crown rust reaction and basal articulation	1.34	6	.98-.95	3.78	6	.80-.70
Crown rust reaction and basal hair length	7.82	6	.30-.20	7.67	6	.30-.20
Stem rust reaction ¹ and heading	2.96	4	.70-.50	5.24	4	.30-.20
Stem rust reaction ¹ and maturity	6.65	4	.20-.10	7.28	4	.20-.10
Stem rust reaction ¹ and basal articulation	9.10	4	.10-.05	3.67	4	.50-.30
Stem rust reaction ¹ and basal hair length	4.85	4	.50-.30	5.68	4	.30-.20
Heading and basal articulation	7.69	4	.20-.10	3.67	4	.50-.30
Heading and basal hair length	6.89	4	.20-.10	11.25	4	.05-.02
Maturity and basal articulation	9.10	4	.10-.05	2.44	4	.70-.50
Maturity and basal hair length	4.85	4	.50-.30	8.40	4	.10-.05
Crown and stem rust reaction ²				9.95	6	.20-.10
Crown rust reaction ² and heading				3.29	6	.80-.70
Crown rust reaction ² and maturity				3.81	6	.80-.70
Crown rust reaction ² basal articulation				9.99	6	.20-.10
Crown rust reaction ² basal hair length				8.35	6	.30-.20
Crown rust ² and smut reaction				6.57	6	.50-.30
Stem rust reaction ² and heading				5.08	4	.30-.20
Stem rust reaction ² and maturity				6.54	4	.20-.10
Stem rust reaction ² and basal articulation				4.23	4	.50-.30
Stem rust reaction ² and basal hair length				1.48	4	.90-.80
Stem rust ² and smut reaction				6.30	4	.20-.10

¹ Mature plant reaction in field.

² Seedling reaction in greenhouse.

SUMMARY

A study was made of the mode of inheritance of reaction to a mixed inoculum of loose and covered smuts, *Ustilago avenae* and *U. levis* crown rust, *Puccinia coronata*, and stem rust, *P. graminis avenae*, for several oat crosses. The inheritance of several kernel characters and earliness and their relation to disease reaction was investigated in the cross Iowa No. 444 \times Bond.

The F_3 distributions for percent of smutted plants indicate that two factor pairs, one a factor for high resistance, the other a factor for partial resistance, govern the inheritance of smut reaction.

The segregation found for crown rust reaction, in the cross Iowa No. 444 \times Bond, suggests the presence of two factor pairs, S a factor for crown rust resistance and I a factor which partly inhibits the expression of S . The masking effect of the inhibitor on the factor S was found to be greater in the mature plant stage in the field than in the seedling stage in the greenhouse. The segregation obtained with

individual races of *Puccinia coronata* was essentially similar to that secured when a composite inoculum was used.

The F_1 seedling reaction indicated a partial dominance of resistance to *Puccinia coronata*, whereas the mature plant reaction in the field showed a partial dominance of susceptibility. In general the agreement between seedling and mature plant reactions was close.

A single factor pair governed the expression of stem rust reaction. Resistance was dominant over susceptibility. A very close agreement was found between seedling and mature plant reaction.

In the cross Iowa No. 444 \times Bond, the characters basal hair length and number, basal articulation, and rachilla attachment were monogenic in their inheritance, while the characters, lemma color, awning, and ratio of kernel width to length were digenic. Linkage was found between all the kernel characters studied.

A partial dominance of earliness was found, as judged by days from emergence to heading and to maturity, for the F_3 of Iowa No. 444 \times Bond and Victoria \times Richland. Significant positive and negative simple correlation coefficients were found between days from emergence to heading correlated, respectively, with days from emergence to maturity, and fruiting period in days.

Smut and crown and stem rust reaction were inherited independent of each other and of the characters' earliness, basal articulation, and basal hair length for the cross Iowa No. 444 \times Bond.

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INJURY TO PEA VINES CAUSED BY THE FEEDING OF THE PEA APHID¹

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INTRODUCTION

The gross effect of aphid feeding on pea plants is easily discernible in the field, but the damage caused to pea vines by the feeding of small numbers of the pea aphid (*Illinoia pisi* Kalt.) has not been fully recognized. It is difficult to distinguish in the field between aphid damage and injury caused by plant diseases and unfavorable climatic or soil conditions. For this reason control measures are seldom used until severe damage has been done.

A more comprehensive knowledge of the injury caused by aphid feeding would be useful in developing a control program and should aid materially in deciding whether or not immediate control measures are necessary at any time in the growth of the plant to prevent injury or complete destruction. To gain more information on the relationships between the growth of the plant, the development of aphid populations, and the extent of injury caused by aphid feeding, a detailed study of the effect of aphid feeding on the pea plant has been made and the data are given in this paper.

MATERIALS AND METHODS

An attempt was first made to carry on this study in the field where a large number of plants were available, but when individual plants were selected for continued observations, weather, fungus, and insect enemies seriously interfered with the work, and satisfactory results were not obtained. Even under greenhouse conditions it was found that keeping records of the aphid infestations and making other daily observations on the plants entailed a time requirement which made it necessary to limit the number of plants to be used, and 60 plants were finally chosen as the unit for the study.

Perfection peas (*Pisum sativum* L.) of the Wisconsin wilt-resistant type were used. The seed was treated with Semesan, Jr., and planted in a mixture of two-thirds compost and one-third sand in 6-inch pots in the greenhouse. Five groups, 12 pots to a group, were planted 1 week apart: the first group was 4 weeks old when the last group was planted. Four seeds were planted in each pot, and about 2 weeks after planting all but one plant in each pot were destroyed; those retained were selected for uniformity in size and vigor.

Young plants did not show extreme differences in size and vigor, but as they grew older such differences appeared, and a number of so-called "strong" and "weak" plants were compared to note the effect of aphid feeding on both types. When the last group of plants was 20 days old from the time of planting, the other groups were respec-

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tively 27, 34, 41, and 48 days old. At this time the height of the plants and the number of internodes were recorded from the growth between the second and the last visible node.

Ten plants were selected to be infested in each group, and two were used as checks. In producing the infestation, one agamic female from a pure line reared on young, uncrowded plants, was placed on the growing tip of each plant before she had started to bear young, and allowed to reproduce for 10 days. All the plants were then covered with 20-mesh screen cages to prevent migration. The tendrils were removed from the plant to prevent their clinging to the cages and disturbing the aphids when the cages were removed. This procedure was followed in order to establish a similar infestation on each plant so that the only variable would be the age and size of the plant. To maintain the plants in suitable condition, they were supported on wooden stakes and watered every 4 days.

No satisfactory method was known for determining the maximum infestation that a plant could withstand and still recover when the aphids were removed. As a rule, as soon as a plant became overcrowded or started to wilt, the older aphids grew restless and began crawling aimlessly about. If the aphids were not removed and counted as soon as this condition developed, an accurate count could not be obtained. If the aphids were removed at this time the plants recovered and could be held for further observation.

This study was carried on between December 24, 1938, and March 27, 1939. The average temperature for the entire period was approximately 60° F. with variations from 55° to 70°, the higher temperatures occurring daily between 10 a. m. and 2 p. m. The relative humidity was approximately 40 percent. With these temperature and humidity conditions, the maximum period that infestations could remain on plants without loss of aphids or plants was determined to be approximately 10 days. This period was arbitrarily set for the removal of all aphids from each plant. At the time the aphids were removed, the height of each plant and the number of internodes were recorded. The plants were retained for continued observations, and additional records were made weekly. A final record of the length of each internode was made at the end of the experiment.

A photographic record of the condition of these plants was made just before the aphids were removed, and a similar record was made of the same plants 18 days later. From these photographs, a series has been selected and presented here to aid in the discussion of the data.

EXPERIMENTAL DATA AND INTERPRETATION

GENERAL EFFECT OF APHID FEEDING

The general effect of aphid feeding was indicated by a wilting of the stipules and leaves at the point of infestation. If the aphid population was sufficiently large and was not removed, individual plants were soon destroyed. If the infestation was removed at the end of 10 days, the plants were stunted only. Weaker plants showed a quicker response in terms of injury than the stronger plants, and younger plants showed the injurious effect of aphid feeding sooner than older plants. The extent of injury appeared to be directly proportional to the number of aphids present and indirectly proportional to the vigor and size of the plant. While the number of plants used for the study

consisted of only 60, with 12 checks, the general effect of feeding was the same for the entire group, with some variations between plants of different age and vigor. As will be shown later, the detailed injury caused by aphid feeding could be noted easily when the aphids were removed and the plants allowed to recover. The stunting of pea plants by aphid feeding was easily duplicated by growing plants in a nutrient solution and substituting water for the solution for a number of days. When the plant was starved except for water, it was only stunted during the period of starvation. However, that portion of the plant fed on by aphids was not only stunted, but the stipules and leaves became shriveled and dried as a result of the removal of water as well as other plant nutrients.

TABLE 1.—*The effect of aphid feeding on the growth of pea plants 20, 27, 34, 41, and 48 days old, when a single agamic female and her progeny were allowed to remain on the plant for 10 days*

Item	Age of plants	Average number of internodes per plant		Difference between number of internodes of noninfested and infested plants	Average height of plants		Difference between height of noninfested and infested plants	Average progeny produced by 1 adult aphid in 10 days			
		Check	Infested		Check	Infested		Adults	Fourth	Other young	Total
	Days	No.	No.	No.	Cm.	Cm.	Cm.	No.	No.	No.	No.
At start of infestation.....	20	3.0	3.12	-----	4.5	5.4	-----				
At removal of infestation.....	30	6.5	6.2	-----	13.25	11.5	-----	1.1	19.9	55.4	76.4
Increase.....		3.5	3.08	-----	8.75	6.1	-----				
Difference.....				0.42			2.65				
At start of infestation.....	27	5.0	5.4	-----	11.5	10.9	-----				
At removal of infestation.....	37	9.0	7.9	-----	20.25	16.6	-----	2.3	21.9	62.1	86.3
Increase.....		4.0	2.5	-----	8.75	5.7	-----				
Difference.....				1.5			3.05				
At start of infestation.....	34	7.0	7.5	-----	15.5	19.9	-----				
At removal of infestation.....	44	10.5	10.5	-----	23.5	25.5	-----	3.5	18.8	61.2	83.5
Increase.....		3.5	3.0	-----	8.0	5.6	-----				
Difference.....				.5			2.4				
At start of infestation.....	41	10.0	9.7	-----	30.5	28.0	-----				
At removal of infestation.....	51	14.0	12.9	-----	45.5	37.5	-----	11.8	17.6	57.2	86.6
Increase.....		4.0	3.2	-----	15.0	11.5	-----				
Difference.....				.8			3.5				
At start of infestation.....	48	11.5	11.3	-----	31.5	31.3	-----				
At removal of infestation.....	58	15.0	14.3	-----	44.0	40.3	-----	7.4	19.2	50.1	76.7
Increase.....		3.5	3.0	-----	12.5	9.0	-----				
Difference.....				.5			3.5				

Table 1 shows the average number of aphids present on each group of plants when the infestation was removed. The adults shown for each age group include the original female and those of her young that had reached maturity but had not started to reproduce at the time the aphids were removed. Just why a larger number of the progeny reached the adult stage in the older groups of plants is not known. It may have been due to a better food supply in the larger plants, as the outside environment of all plants was the same. The writers cannot account for the difference in number of adults in the two oldest groups shown.

Some variation occurred in the number of aphids per plant in each age group. There was also a difference in the total average number of aphids per group, but it is thought that these variations caused no significant difference.

The general effect of aphid feeding on pea plants is shown in a series of photographs in figure 1. *A* shows two plants 30 days old; the plant at *a* was infested when 20 days old, the plant at *b* was not infested. *B* shows the same two plants 18 days after the aphids were removed. It will be noted that the number of internodes on the two plants (fig. 1, *B*) is practically the same, but the infested plant at *a* is stunted. Figure 1, *C* and *D*, shows two other plants treated in the same manner; the plant at *C*, *a* was infested when 41 days old. *D* shows the same two plants 18 days after the aphids were removed. The infested plant at *b* blossomed a week later than the noninfested plant at *a*.

DETAILED EFFECT OF APHID FEEDING ON STIPULES, LEAVES, AND INTERNODES

Although stipules and leaves may be destroyed and internodes shortened, a detailed study of aphid feeding showed that if the aphids were removed before the plant was completely destroyed, it could recover and grow to maturity. Aphid feeding stunted or destroyed the stipules and leaves at the point of infestation but did not stop the growth of the plant (fig. 2).

Aphid feeding caused a shortening of the internodes during the time of infestation, but as soon as the aphids were removed the internodes began to lengthen.

The internodes of the younger plants were shortened more than those of the older plants. The diameter of the stem in each case was also reduced by aphid feeding; that is, the part of the stem that grew before or after the infestation was larger than the part that grew during the infestation. The internodes of weak, infested plants were shortened much more than those of stronger plants of the same age (fig. 1, *E*).

A graphic presentation of the effect of aphid feeding on the internodes of different-aged groups of plants and a comparison between the internodal growth of the infested and noninfested plants are shown in figure 3. In each age group the average length of 10 consecutive internodes for infested and noninfested plants is indicated. In each graph the broken lines represent the average length of internodes from noninfested plants and the solid lines the average length of internodes from infested plants. Internode No. 1 in each graph represents not the length of the first internode of the plants but the length of the second internode below the point of infestation. Line *a* represents the normal growth of 10 consecutive internodes from noninfested plants, and line *b* the corresponding internodes from infested plants. *x-x* in line *b* marks the limits of the 10-day infestation.

It was observed that in practically every case the shortening of the internodes began immediately after the plants were infested, showing that the feeding of only a few aphids affected growth. It is also to be noted that as soon as the aphids were removed, the internodes began to lengthen in the plants of all ages, but not to the same extent for each group. In the 20- and 27-day-old groups, the infested plants were slow to recover as was shown by the slow increase in the length of the internodes as compared to those of the noninfested plants. The 20-day-old group was even slower in recovering than the 27-day-old group.

The 34- and 41-day-old groups made good recovery, as expressed by increase in length of internodes. The final internode was even slightly longer in the infested plants than in the noninfested plants.

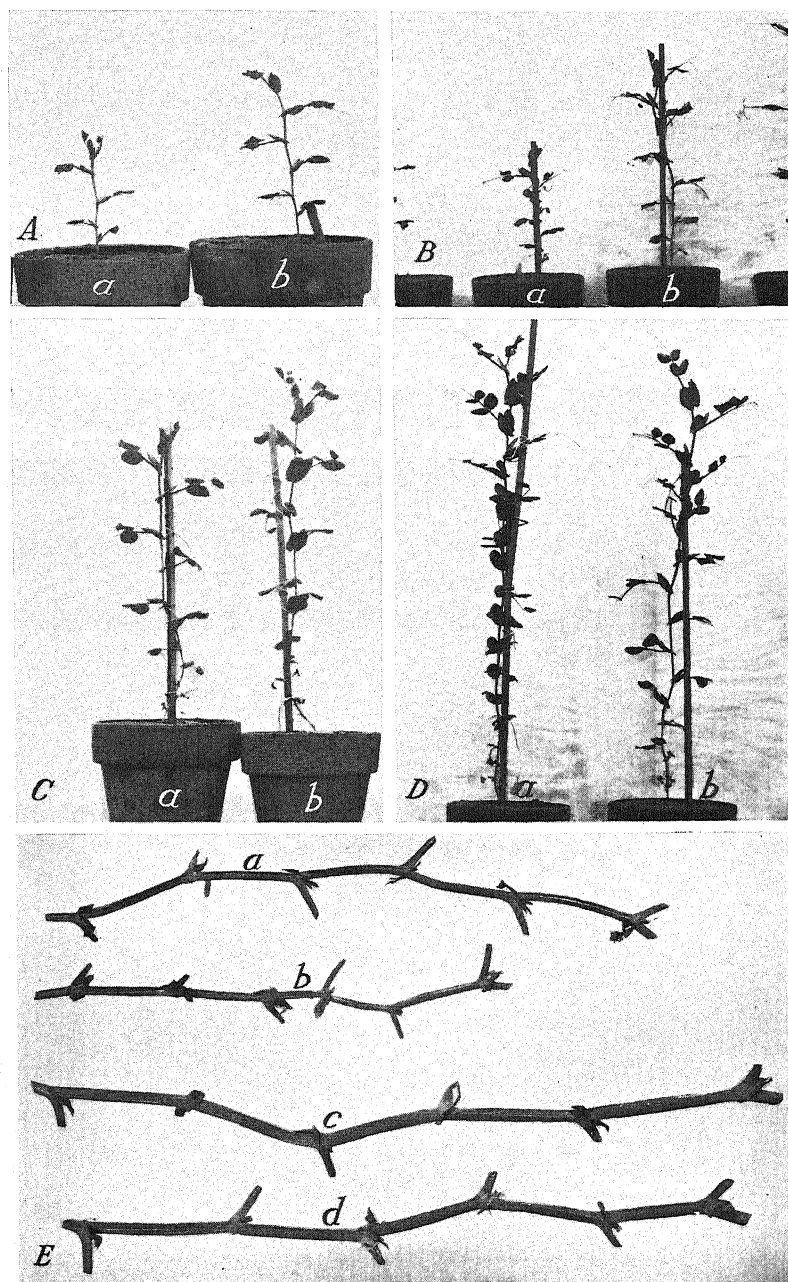


FIGURE 1.—Effect of aphid feeding on pea plants: *A*, Pea plants 30 days old: *a*, Infested when 20 days old with one agamic female aphid; *b*, noninfested. *B*, Same plants as in *A* 18 days after aphids were removed. *C*, Plants 51 days old: *a*, Infested when 41 days old with one agamic female aphid; *b*, noninfested. *D*, Same plants as in *C* 18 days after aphids were removed; infested plant at *b*. *E*, Sections of pea plants of the same age but of different vigor showing internodes shortened by aphid feeding: *a* and *c*, Normal-length internodes; *b* and *d*, internodes shortened by aphid feeding. Aphid feeding injures weak plants *a* and *b* more than strong plants *c* and *d*.

In the 48-day-old group the infested plants showed good recovery for several internodes only and then leveled off. This leveling off in internode length before the length of the noninfested plants was

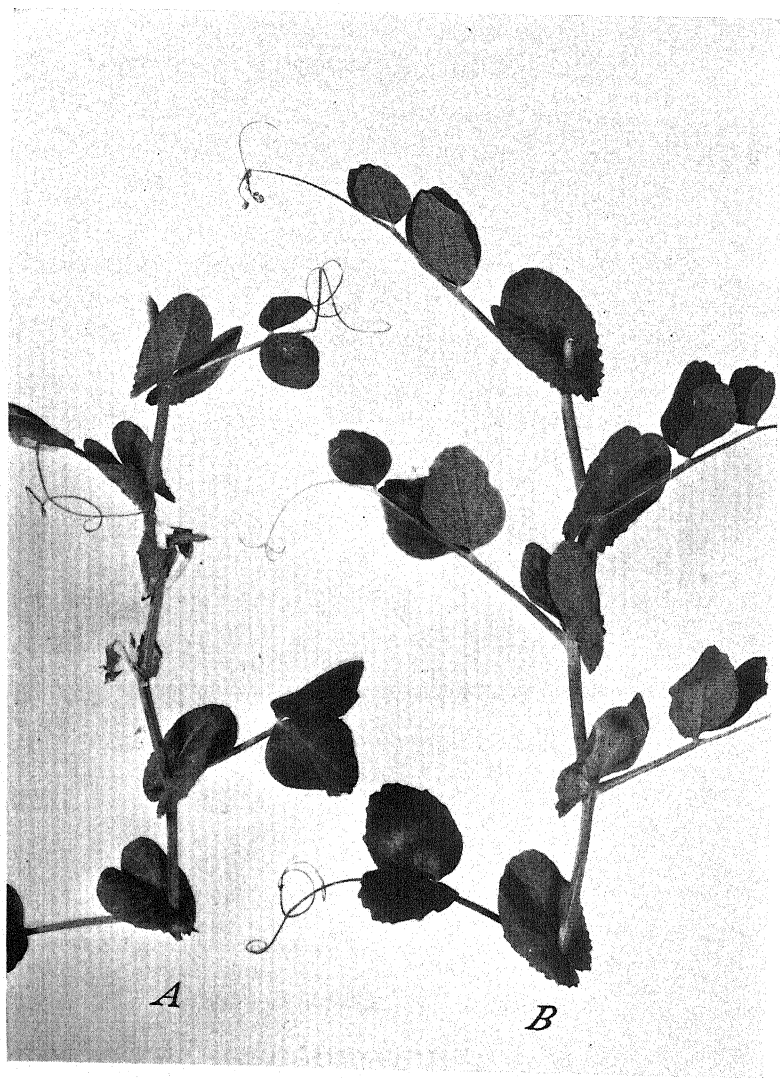


FIGURE 2.—Aphid feeding causes a shortening of internodes and destruction of stipules and leaves during the time of infestation. Stipules and leaves at several subsequent nodes are also reduced in size: A, Plant infested with one agamic female when 44 days old and female and progeny removed 10 days after infestation; B, noninfested plant of same age as that in A.

reached may have been due to the fact that the infested plants bloomed at about this time. As shown in the all-plant average, the infested plants recovered well and finally the length of the internodes equaled that of the noninfested plants.

There was also a tendency toward a slight reduction in the number of internodes produced in an infested plant as compared with that produced in a noninfested plant in the same length of time. The differences for each age group are shown in table 1. It was noted that the leaves and stipules of subsequent nodes were also reduced in

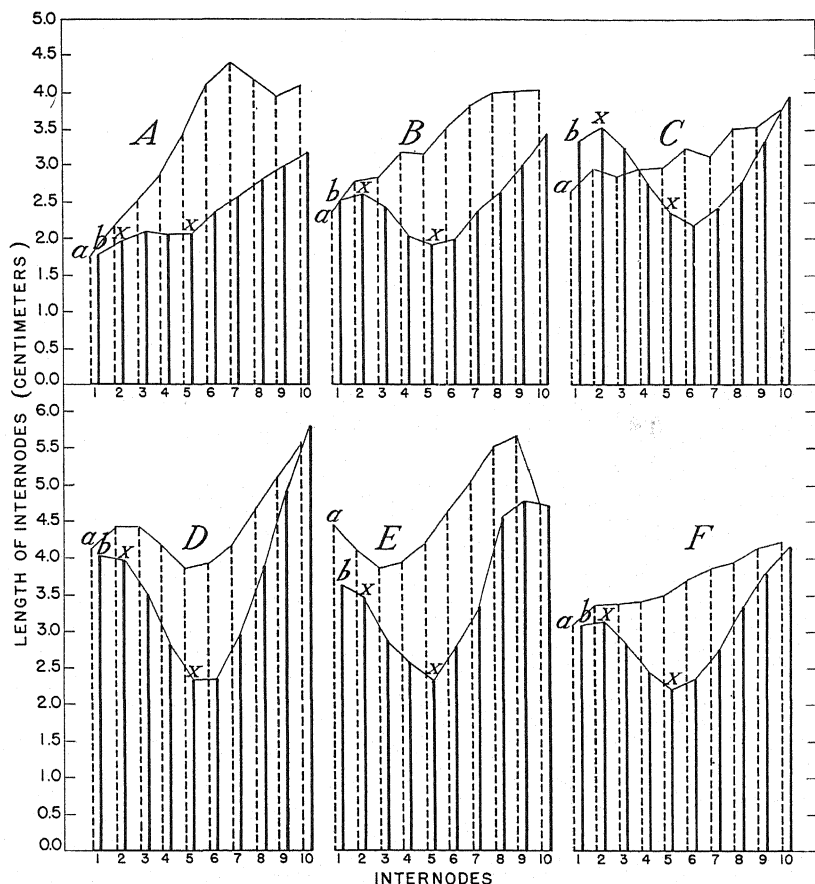


FIGURE 3.—Shortening of the internodes of pea plants of different ages caused by aphid feeding. Plants infested when: A, 20 days old; B, 27 days old; C, 34 days old; D, 41 days old; E, 48 days old. The all-plant average is shown in F. Normal internodal growth of noninfested plants indicated by a, of infested plants by b, and duration of infestation by x-x. Figures 1 to 10 at the bottom of each graph represent consecutive internodes. Broken lines represent average length of internodes from noninfested plants and solid lines average length of internodes from infested plants.

size, but reached a more normal size at the later nodes. The stunting of these parts of the pea plant, as indicated before, undoubtedly was due to the removal of plant sap.

The smaller plants were more affected by the aphid feeding than the larger plants. This indicates that an equal population of aphids on a series of plants removed about the same amount of food material, thus demonstrating a difference in the ability of plants of different size and vigor to withstand a loss of plant sap. It is logical to reason that if an

equal amount of water and food were removed from small or weak plants and strong vigorous plants, the injury would be more pronounced on the former. The small plant would also be more stunted and would recover more slowly because of the reduction of leaf surface available for food manufacture.

In table 1 the data show the average number of internodes and height for each age group of plants at the beginning of the infestation and at the time the aphids were removed. The average differences may not appear great for a period of 10 days, but, as shown in figure 1,



FIGURE 4.—Aphid feeding on pea plants near the blossom stage delays blossoming. Two plants of the same age: A, Noninfested plant with normal growth and podding; B, infested plant on which the first blossom did not appear until 2 weeks after the first blossom on plant shown in A.

B and D, the injury was very pronounced 18 days after the aphids were removed.

INJURY TO BLOSSOMS AND PODS

In feeding, many species of aphids move from the older and less succulent parts of the plant to the new growth. This is true of the pea aphid, and the greatest population is usually found on the growing tip. When a dozen or more reproducing mothers occur simultaneously on a plant, the increase in population may be such as completely to cover both stem and leaves on the upper portion. As the pea plant develops and the bud cluster opens, aphids settle on the buds, and each blossom pedicle may have a row of aphids feeding along its entire length. Frequently the flower part is also infested inside and out, and quickly withers.

It was interesting to observe how aphid feeding affected the blossoming of plants infested at different stages of growth. When plants 20 and 27 days old were infested at the growing tip with a single reproducing female and the aphids were allowed to remain 10 days, the plant blossomed at about the regular time and at the regular node.

In the plants infested when 34 days old and the aphids removed after 10 days, the blossoms appeared earlier than those on the noninfested plants.



FIGURE 5.—Aphid feeding on pea plants stimulated development of axillary buds below the feeding area several weeks after the aphids were removed: *A*, Injury to growing tip of budding plant stimulated development of flower buds at lower nodes; *B*, similar injury to young plants caused stooing at lower nodes.

Plants infested when 41 days old and the infestation removed after 10 days, blossomed 1 week later than the check plants. Plants infested when 48 days old and treated in the same manner did not blossom until 2 weeks after the check plants (fig. 4).

When plants were infested at the beginning of the blossom stage, no pods were produced during the time of the infestation. Also, it was found that when plants were infested at the top after one or more pods had formed, additional blossoms were blighted and no more pods formed. When the growing tip became overcrowded with aphids, some moved on to the pods and a "crippled" condition developed. The extent of damage was proportional to the number of aphids present.

DEVELOPMENT OF ADVENTITIOUS BUDS

It was found that aphid feeding on the growing tip of pea plants sometimes caused the development of adventitious buds (fig. 5). This condition was not general and probably appeared only when the growing tip was severely injured.

When the tops of young plants were injured, they developed vegetative growth at the axes of the lower leaves (fig. 5, *B*). This condition also develops in the field when plants are injured by frost or other agencies.

Older plants, infested a short time before bloom, produced flower buds at the axes of the upper leaves (fig. 5, *A*). These did not appear until the plant had blossomed at the tip. These adventitious blossoms may have been those that were inhibited at the normal time because of the aphid infestation. These blossoms dropped before or soon after podding.

SUMMARY

A study of the effect of aphid feeding on pea plants was made in the greenhouse to obtain a definite knowledge of the injury caused to pea vines by a controlled aphid population. Plants of five age groups were used for the study and each of these was infested with one nearly mature adult aphid. Each infestation was allowed to remain on the plant for 10 days. Suitable check plants were maintained.

The injurious effect of aphid feeding on pea plants of different ages and vigor is believed to be due to a withdrawal of plant sap. When a single agamic female and her progeny were allowed to remain on any plant for 10 days, definite injury to plants of all ages was demonstrated. If the aphids were not removed as soon as the plant began to wilt, it was soon destroyed. As a result of aphid feeding, the stipules and leaves were reduced in size or destroyed and the internodes were shortened in the area occupied by a limited infestation of aphids. The extent of the injury to pea plants was directly proportional to the number of aphids present. With comparable infestations the injury was more pronounced on younger than on older plants, and on less vigorous than on strongly growing plants. Aphid infestations on older plants caused a delay in the blossoming period of as much as 2 weeks.

Under the controlled conditions of temperature and moisture maintained in these experiments it was found that if a single agamic female was placed on a plant at blossom time and before the pods were set, she and her progeny could cause complete destruction of the blossoms and prevent the development of the pods.

RESISTANCE TO CLUBROOT IN VARIETIES OF TURNIP AND RUTABAGA¹

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INTRODUCTION

Clubroot (*Plasmodiophora brassicae* Wor.) continues to be a major disease of crucifers in many countries. Although it has been known for several centuries and has been the subject of numerous scientific investigations during the past 60 years, entirely satisfactory remedial measures have not been worked out for the important crops on which it occurs. Because of the extreme longevity of the pathogen in the soil the disease becomes one of that group in which control is best obtained through the development of disease-resistant varieties. This report concerns one of a series of studies under way on the occurrence and nature of clubroot resistance in the crucifers. The subject has been advanced farthest in the case of the turnip and rutabaga,² which are the two crops considered in this paper.

PREVIOUS WORK ON RESISTANCE TO CLUBROOT IN TURNIP AND RUTABAGA

As early as 1853, Anderson (1)³ noted in England that white-fleshed turnips were more subject to clubroot than yellow-fleshed ones. In 1894 Rostrop (30) in Denmark stated that rutabagas were more resistant than turnips. No other comments on this subject seem to have been recorded until the present century. In 1902, in a note in the Journal of the Board of Agriculture of Great Britain (11, p. 149), it was stated with reference to this disease as follows: "Of late years several so-called disease-proof turnips have been put on the market, and though all are certainly not immune from disease, some are markedly resistant."

The first critical tests of varietal resistance to clubroot in turnip and rutabaga were carried out in 1912 and 1913 in Vermont by Cunningham (5). He tested 10 varieties of turnip and the same number of varieties of rutabaga on heavily infested soil in the field. The Southern Curled variety of turnip had 100 percent of infected plants, whereas Early White Milan, Large Amber Globe, Early White Flat Dutch, Strap Leaf, White Egg, Yellow Stone, and Early Snowball each had less than 10 percent of diseased plants. Other varieties tested were between these two extremes. Among the

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² The term "turnip" has been used by some to refer to members of both *Brassica rapa* L. and *B. napobrassica* Mill. (*B. campestris* L. var. *napobrassica* DC.). In this paper it is used to refer only to varieties of *B. rapa*. The term "rutabaga" is applied to varieties of *B. napobrassica* which are also commonly referred to in the literature as "swedes" and sometimes erroneously as "yellow turnips."

³ Italic numbers in parentheses refer to Literature Cited p. 825.

rutabaga varieties there was also a wide range of susceptibility. One variety from India had 95.7 and 95.4 percent of infected plants in 1912 and 1913, respectively. At the other extreme were White Swede, Sweet German, Purple Aberdeen, and Sweet Russian, which showed either no signs of disease or less than 1 percent of the plants infected.

In 1917 Christensen (4) in Denmark stated that the English variety Pioneer was highly resistant to clubroot. He also reported the results of a program of improvement in resistance which he had started in 1908 with healthy survivors from two local varieties grown on heavily infested soil. From these, several families were developed that were even more resistant than the variety Pioneer. Two of these families (4 and 25) were tested on infested soil in Wales by Whitehead, who reported them in 1922 (36) and again in 1925 (37) as distinctly more resistant and productive than other varieties under observation. Family 4 was later introduced by Christensen as Studsgaard Bangholm, while Wilhelmsburger, another resistant variety of rutabaga, was introduced from Denmark about the same time.

In 1924 and 1925 Lindfors (22, 23) reported extensive trials of varieties of turnip and rutabaga on clubroot-infested soil in Sweden. Of the fodder turnip varieties he found Svalöf's Yellow Tankard, Dale's Hybrid, Weibull's Sekel, and Weibull's Östersundom the most resistant. Of the rutabaga varieties Studsgaard Bangholm, Wilhelmsburger, Svalöf's Yellow Swedish, and Weibull's Swedish Smooth were the most resistant, while Svalöf's Bangholm, Weibull's Bangholm, Weibull's Imperial, and Weibull's Trondhjem were very susceptible. None of the turnips or rutabagas was completely resistant, however, for even the Studsgaard Bangholm was completely destroyed by the disease on poorly drained, acid soil.

The importance of fodder turnips and rutabagas and the severity of clubroot in the Maritime Provinces, Canada, led to studies of varietal resistance in Nova Scotia by Hockey (14) and in New Brunswick by MacLeod (25; 27, *Rpt.* 1930). In Nova Scotia, tests of many varieties showed a high percentage of infected plants in all, but the Herning and Studsgaard strains of Bangholm were usually the least seriously affected by the disease. In New Brunswick, similar results were secured. Selections were made from several strains of Bangholm which were highly resistant. One selection from White Swede, a variety found by Cunningham in Vermont (5) to be highly resistant, maintained a high degree of resistance through several generations. A cross between the selection from White Swede and Studsgaard Bangholm gave most promise.

In 1929 Osterwalder (29) reported on trials made in Switzerland in 1922 and 1923, in which three varieties of turnip—Gelbe Schmalz, Weisse Schmalz, and Apfel—remained completely free from clubroot in a planting in which cabbage and cauliflower showed high percentages of infection.

In 1931 the Bruce variety of fodder turnip was reported by Findlay (8) as capable of producing a commercially successful crop on heavily infested land. This variety had been in use in certain localities in Scotland for upward of 100 years. High percentages of infected plants were found to occur, but the fact that the disease was confined almost entirely to the fibrous roots permitted it to make suc-

cessful growth in spite of the disease. A year later Hendrick (13) reported the results of 5 years' trials in Aberdeen County, Scotland, in which Bruce produced satisfactory crops on heavily infested land where other varieties were almost completely destroyed.

Thus, by 1932, the control of clubroot by the use of resistant varieties of turnip and rutabaga had progressed to a marked degree. This was particularly true of the fodder varieties in northern Europe; in New Zealand; and in the Maritime Provinces, Canada, where the culture of these crops comprises extensive acreages. Of the rutabagas, the Wilhelmsburger variety and the Herning and Studsgaard strains of the Bangholm variety continue to be among the most highly resistant (6, 12, 19, 31, 33, 34). The Balmoral variety was reported to be successfully resistant in Devon and Cornwall, England, by Beaumont (3) in 1933. Of fodder turnips the May variety (Norwegian May, Marienlyst Majturnip) has been found resistant in Denmark (28) and in Canada (27, *Prog. Rpt.*). In addition to the reports from Scotland noted above (8, 13), Bruce has been shown to be commercially successful as a resistant variety in Canada (12), in Devon and Cornwall, England (3), and in New Zealand, where a green-topped selection known as Wallace is also satisfactory (34). Dale's Hybrid was recorded as highly resistant in Sweden in a report from Svalöf Plant Breeding Institute (32) in 1929, confirming the earlier report by Lindfors (22); this variety was found to be resistant in Germany also (24). The early strain of Bortfelder, Östersundom, reported to be resistant by Lindfors (22), was listed as moderately resistant at Svalöf in 1929 (32), while the Fünen strain of Bortfelder was reported in 1933 to be resistant in Denmark (28). Irvine's Green-Top Yellow turnip was reported to be resistant in New Zealand (33), while Yellow Tankard, listed earlier (1924) as resistant in Sweden (22), was found to be among the most resistant some 10 years later in eastern Canada (27, *Prog. Rpt.*) and in Finland (18). Lorenzen (24) listed another variety, Crieewener Rübe, as resistant in Germany.

It should be pointed out that the reports of Lindfors (22, 23) in Sweden, Hockey (14) and MacLeod (25; 27, *Rpt. 1930*) in Canada, and Jamalainen (18) in Finland emphasized that under certain conditions so-called resistant varieties showed all or nearly all plants to be infected. Their value lay in the fact that, even in an extremely favorable environment for the pathogen, infection was less damaging to the majority of the plants of resistant varieties, and that under less favorable conditions for infection a much larger proportion of the individuals of the resistant sorts escaped infection. It was also recorded by Findlay (8) that in the case of the variety Bruce, which was shown to be eminently productive on heavily infested soil, a large percentage of plants became infected, but the damage was usually negligible because the clubs were confined largely to the secondary roots while the fleshy taproot remained free from disease.

Eriksson (7) remarked that round varieties of turnips were less subject to clubroot than long ones. While this may apparently be true it is more likely that the real difference between the two groups is that pointed out by Larson (20), who showed that infection of the hypocotyl was relatively rare unless it was wounded. Thus in the round or globe varieties in which the fleshy portion consists chiefly

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of enlarged hypocotyl, the incidence of infection and clubbing is quite low as compared with that in the long varieties in which the lower portion of the enlarged storage organ is taproot supporting two zones of fibrous secondary roots through which the fleshy taproot is readily invaded by the clubroot pathogen.

The importance of variation in resistance among species and varieties of the host cannot be finally and satisfactorily evaluated without a knowledge of the variability of the pathogen. The question of physiologic races within the species *Plasmodiophora brassicae* has been raised by a number of investigators. It was suggested by Appel and Werth (2) in 1910, by Höstermann (15) in 1922, and by Gleisberg (10) in 1923, but in no case was definite evidence of this type of specialization presented. In 1931 Gibbs (9) reported that he had found no evidence of the existence of physiologic races in New Zealand. In the same year MacLeod (26) reported studies in New Brunswick, Canada, wherein inocula collected from a number of cultivated and wild hosts showed no pathogenic differences when used to inoculate a number of varieties of turnip and rutabaga. However, in a later note the same writer (26, *Prog. Rpt.*) reported marked differences in infection of several species and varieties of crucifers when grown on infested soil collected from seven different locations in the Maritime Provinces. Also in 1931 Honig (16) reported studies on this subject from Germany. He collected inocula from kohlrabi (*Brassica oleracea* L. var. *caulo-rapa* DC.), cauliflower (*B. oleracea* var. *botrytis* L.), white and savoy cabbage (*B. oleracea* var. *capitata* L.), and from radish (*Raphanus sativus* L.) and studied the degree of infection which resulted when each of these was used to inoculate clubroot-free soil upon which several cruciferous species were grown. The collections from kohlrabi and cauliflower, presumably similar, caused heavy infection on kohlrabi, cauliflower, and *B. nigra* Koch. One of these (kohlrabi collection) was tested on *Camelina sativa* Cr., on which it caused a high percentage of infection. Both inocula caused very low percentages of infection on *Raphanus sativus* and *R. oleiferus*. The collection from savoy cabbage produced heavy infection on *R. oleiferus* but little on *B. nigra*. The inoculum from white cabbage caused a high percentage of infection in *R. oleiferus*, an intermediate percentage on white cabbage and kohlrabi, and a low percentage in *Camelina*. The collection from *R. sativus* infected a low percentage of plants of *Camelina* and of *B. nigra* and a high percentage of plants of *R. oleiferus*. In a later paper Honig (17) reported percentages of infection of turnip varieties as follows: Gelbe Schmalz, 7.3; Weisse Schmalz, 27.7; Weisse Wester, 0; Gelbe Wester, 32.6; Apfel Gelbe, 6.2. He pointed out that the first two of these varieties had been reported earlier by Osterwalder (29) from Switzerland as completely free from infection on soil where cabbage and cauliflower became heavily clubbed. On the basis of his results, Honig concluded that physiologic races of *P. brassicae* occur. In 1936 Whitehead (38) suggested a similar explanation for the fact that in certain of his experimental plots a high percentage of plants of white and savoy cabbage and brussels sprouts became clubbed, whereas varieties of rutabaga which were usually heavily infected in that region were in this case only slightly affected.

SCOPE OF THE PRESENT INVESTIGATION

No critical study of the range of resistance in varieties of turnip and rutabaga used in the United States has been made since the work of Cunningham (5) about 25 years ago. The present investigations were started in conjunction with field studies upon the control of clubroot on cabbage by soil treatment, reported upon elsewhere (21, 35). The object of the work was to collect the varieties now in common use in the United States and determine what range in resistance occurs among them.

METHODS AND MATERIALS

The investigation was begun with trials conducted at Franksville, Racine County, Wis., in a portion of the experimental field already described (21) in which a high percentage of clubroot occurred on cabbage. The field trials at Franksville were supplemented by similar trials at Madison, Wis., in which soil from the Franksville field was used for inoculum.

Inasmuch as the clubroot organism is very sensitive to changes in soil moisture and reaction, it was found that field trials, though highly indicative, were not entirely reliable, since uncontrollable changes in environment sometimes led to the escape of many susceptible individuals from infection. Greenhouse tests were therefore used extensively as the investigations progressed and eventually were relied upon almost entirely for the final evaluation of a given sample. Muck soil from a heavily infested field a few miles from the Franksville plot was used in the greenhouse studies. For these trials plants were grown upon noninfested soil until the second or third leaf had unfolded, when they were transplanted to infested soil.

In recording disease development at the end of a given experiment, plants were divided into two classes—healthy and diseased—determined by the presence or absence of macroscopic signs of infection. No attempt has been made in the results reported herein to determine grades or degrees of infection within the "diseased" group.

In the course of the greenhouse tests it was found that as the soil was used for repeated trials it tended to become less infectious. At first this was interpreted as a gradual depletion of the pathogen. Examination of the soil showed that, although it was quite acid in reaction (around pH 5.2) when first used, it tended to change toward alkalinity rather rapidly under greenhouse conditions. When it was brought back to pH 5.2 to 5.6 by the addition of dilute sulfuric acid its high infectivity returned.

In view of the fact that physiologic strains of the parasite may exist and thus have a direct influence upon the extent and type of resistance in the host, it was considered desirable to include collections of *Plasmodiophora brassicae* from widely separated localities. Soil or diseased plants from many diseased areas were therefore secured. When only diseased plants were obtained, the macerated clubs were incorporated into acid soil and susceptible plants were grown upon it. Clubs from repeated planting were added to the soil until it became highly infectious. The trials on soils containing the various inocula were all conducted in the greenhouse.

The seeds used in these experiments were all collected from commercial sources.

EXPERIMENTAL RESULTS

TURNIP VARIETIES

The results with turnip in both field and greenhouse are brought together in table 1. The field experiments were carried on in 1932, 1933, and 1934, while the greenhouse tests were made in 1935, 1936, and 1937. The severity of each test may be estimated best by the percentage of infection in the most susceptible variety, Shogoin. Only in the 1934 trial at Franksville was the proportion of diseased plants unusually low for this variety.

Certain varieties remained free from signs of the clubroot disease in all of the trials reported in which they were included. These were May, Snowball, Purple Top Milan, Golden Ball, Rhode Island Rock, Yellow Aberdeen, and Immuna. The last-named variety was developed at and released recently by the Weibull Plant Breeding Institute at Landskrona, Sweden.

TABLE 1.—Occurrence of clubroot upon varieties of turnip in field trials at Franksville and Madison, Wis., and in greenhouse trials with naturally infested soil from Racine County, Wis.

[illegible]

TABLE 1.—Occurrence of clubroot upon varieties of turnip in field trials at Franksville and Madison, Wis., and in greenhouse trials with naturally infested soil from Racine County, Wis.—Continued

Sample No.	Variety	Greenhouse trials in Racine County soil									
		1935 No. 1		1936 No. 1		1936 No. 2		1936 No. 3		1937 No. 1	
		Plants tested	Plants diseased	Plants tested	Plants diseased	Plants tested	Plants diseased	Plants tested	Plants diseased	Plants tested	Plants diseased
		No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
22	May	70	0.0	303	0.0	225	0.0				
113	Snowball	67	.0	178	.0	142	.0	157	0.0	87	0.0
142	do	30	.0								
154	Purple Top Milan	68	.0	120	.0			183	.0		
155	White Milan	66	.0	54	.0			105	.0		
146	Golden Ball							175	.0		
134	Rhode Island Rock			24	.0						
147	Yellow Aberdeen							58	.0		
200	Immuna									96	.0
143	White Egg	68	5.9					178	2.8		
152	Seven Top	66	10.6								
135	do	61	14.8					178	6.7		
151	White Norfolk	58	.0					175	5.1		
139	Pomeranian White Globe	54	9.3	58	8.6			186	7.0		
116	Purple Top White Globe	67	3.0								
156	do	66	6.1								
136	do	57	1.8								
137	do	63	5.0								
145	Amber Globe	68	7.4					167	10.2		
117	Purple Top Strap Leaf	65	30.8					179	23.5		
138	do	44	34.9								
144	White Flat Dutch	68	16.2					174	13.8		
114	Cowhorn	69	40.6					176	25.0		
140	do	56	39.3								
141	Bortfelder	68	36.8	89	29.2						
3991	Earliest White Flat							228	74.1		
153	Di Rapi							123	51.2		
115	Shogoin	66	100.0	96	95.8	132	100.0	153	83.0	76	93.7
5503	do					52	98.1	170	97.6		

In another group of varieties consisting of White Egg, Seven Top, White Norfolk, Pomeranian White Globe, Purple Top White Globe, and Amber Globe, no infected plants occurred in some of the tests; but in other tests a small percentage of the plants, usually less than 10 percent, were diseased.

Purple Top Strap Leaf, White Flat Dutch, Cowhorn, Bortfelder, and Earliest White Flat each showed a considerable percentage of infected plants. The severest test was undoubtedly that conducted in the greenhouse in 1935 (designated as 1935 No. 1 in the table). Except for White Flat Dutch, which had only 16 percent of infected plants, and Earliest White Flat, which was not included in this trial, the percentage of diseased individuals for varieties in this group was 30 percent or higher. In the 1937 No. 1 trial Earliest White Flat had 74 percent infection. These varieties obviously contain a proportion of susceptible plants large enough to sustain considerable losses in clubroot-infested soil.

Di Rapi showed a very high percentage of infection in two trials. It appears to be in the highly susceptible class with Shogoin. Two seed samples of the latter showed 80 percent or more of infected plants in each test in which one or both strains were included except in the 1934 trial at Franksville, where environmental conditions undoubtedly were responsible for the low incidence of disease.

RU TABAGA VARIETIES

The rutabaga trials ran concurrently with those of turnip in both field and greenhouse. The results are presented in table 2. The data from susceptible Shogoin turnip are given in those instances in which it was included. The outstanding feature of the rutabaga trials is the absence of infection in most varieties and the uniformly low percentage of infection in the remaining ones. Under the same conditions Shogoin turnip was heavily infected. It was not surprising to find this low incidence of disease in such varieties as Studsgaard Bangholm and Wilhelmsburger, which have been found to be highly resistant in other regions. What was unexpected was the rarity of clubbed plants in such varieties as Model, Lord Derby, Tipperary, and Magnificent, which are regarded as very susceptible in Wales. In fact, the seed samples of these varieties were secured from T. Whitehead, of Bangor, Wales, who had found them to be among the most susceptible sorts in that region. It is also to be recalled that Hockey (14) in Nova Scotia and MacLeod (25; 27, *Rpt. 1930*) in New Brunswick found these and many other varieties of rutabaga to be very susceptible in those areas. These results again raise the question of the occurrence of physiologic races of the pathogen, inasmuch as a generally high resistance of rutabaga varieties has not been reported elsewhere.

TABLE 2.—*Occurrence of clubroot upon varieties of rutabaga in field trials at Franksville and Madison, Wis., and in greenhouse trials with naturally infested soil from Racine County, Wis.*

[illegible]

TABLE 2.—Occurrence of clubroot upon varieties of rutabaga in field trials at Franksville and Madison, Wis., and in greenhouse trials with naturally infested soil from Racine County, Wis.—Continued

Sample No.	Variety	Greenhouse trials in Racine County soil											
		1935 No. 1		1935 No. 2		1936 No. 1		1936 No. 2		1936 No. 3		1937 No. 1	
		Plants tested	Plants diseased	Plants tested	Plants diseased	Plants tested	Plants diseased	Plants tested	Plants diseased	Plants tested	Plants diseased	Plants tested	Plants diseased
		No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
15	Wilhelmsburger.....	48	0.0			62	0.0	280	0.0	113	.0		
165	do.....	52	.0	95	0.0							10	0.0
110	White Russian.....	52	.0	110	.9							80	.0
111	White Neck'less.....	45	.0			234	1.7	222	.9			30	.0
148	do.....	15	.0	17	.0								
112	American Purple Top.....												
149	do.....												
150	do.....												
132	Monarch.....	47	.0	91	.0					61	.0		
131	Lain's Swede.....	51	.0	28	.0					109	.0		
162	Lord Derby.....	53	.0	79	1.3							57	.0
163	Model.....	54	.0	73	4.1							10	.0
164	Tipperary.....	53	.0	89	.0							37	.0
161	Magnum Bonum.....	46	.0	106	.9							17	.0
160	Magnificent.....	51	.0	89	2.4							43	.0
16	Studs-gard Bangholm.....	51	.0	58	.0	238	.0	245	.0	111	.0		
133	Carter's Imperial.....	51	2.0	131	2.3					112	.0	19	.0
166										105	.0		
115	Shogoin Turnip (control).....									111	92.8		
1												15	.0
2												70	.0
3												53	.0

REACTION OF TURNIP AND RUTABAGA TO INOCULA FROM DIFFERENT REGIONS

The results reported so far were secured from inoculum coming originally from a single county in Wisconsin, and the question of regional variation of the clubroot organism as to its pathogenic selectivity naturally arose. To obtain information on this point inocula were secured in the form of naturally infested soil or as diseased plants from Indiana, Michigan, New York, New Jersey, Massachusetts, and Washington State. When diseased plants were secured the diseased roots were macerated and incorporated into acid muck soil. Several successive crops of susceptible turnip were then grown and in each case the diseased roots were macerated and returned to the soil until it had become highly infectious.

The most susceptible turnip, Shogoin, and Snowball, the one that had remained healthy consistently in the infested Wisconsin soils, were planted in each of the infested soils. The results in table 3 show that Shogoin was readily and quite thoroughly infected by each inoculum. Snowball remained free from infection in all soils except two. Four out of 114 plants were infected in the Indiana soil, while in the soil inoculated from plants received from Washington State 12.2 percent became infected. The American Purple Top rutabaga, which had remained completely free from disease in the Wisconsin field tests, showed no infection in the New York soil, while 10.3 percent of the plants were clubbed in the New Jersey soil.

TABLE 3.—Occurrence of clubroot on Snowball and Shogoin turnip and American Purple Top rutabaga when grown in the greenhouse upon soil infested with inoculum from various States

Source of inoculum	Shogoin turnip		Snowball turnip		American Purple Top rutabaga	
	Plants tested	Plants diseased	Plants tested	Plants diseased	Plants tested	Plants diseased
	Number	Percent	Number	Percent	Number	Percent
Wisconsin.....	76	98.7	87	0.0
Indiana.....	51	98.0	114	3.5
Michigan.....	28	100.0	22	.0
New York.....	45	100.0	49	.0	20	0.0
New Jersey.....	21	76.2	23	.0	39	10.3
Massachusetts.....	24	100.0	30	.0
Washington.....	30	93.3	74	12.2

DISCUSSION

The trials conducted with rutabaga and turnip varieties in Wisconsin soil show a wide range of resistance and susceptibility to *Plasmiodiophora brassicae* within the two species of *Brassica*. Insofar as turnip is concerned the results are in general accord with those obtained elsewhere. For instance, the May variety was found resistant in Canada and in Europe, while Amber Globe, White Milan, White Egg, and Snowball were found resistant in Vermont by Cunningham. The chief difference between the results obtained by the writer and those of others lies in the fact that in several varieties no infection whatever was secured in clubroot-infested Wisconsin soils. This is contrary to the results of Cunningham, who usually secured some infection on all varieties, and it differs especially from the results in Canada and in Sweden, where under field conditions high percentages of infected plants often occurred among the so-called resistant varieties. Lindfors (23) found that in very acid, poorly drained soil even the resistant forms of rutabaga and turnip were completely destroyed. In the greenhouse trials of this investigation the soil was kept both moist and acid, but this measure did not materially change the results from those in the field nor did it result in any infection whatsoever in the case of several varieties.

The field and greenhouse trials of rutabaga show quite clearly that the inoculum used from Wisconsin soils has little pathogenicity for any of the varieties tested. When this is compared with the incidence of infection on both resistant and susceptible varieties of rutabaga in Canada and in Sweden it must be concluded that either marked differences prevail in the selective pathogenicity of the pathogen in those localities as compared with that in Wisconsin, or some condition of the environment which increases infection in those localities has been overlooked here. While differences in inherent resistance or susceptibility between strains of a given variety are not to be overlooked, it may be pointed out that samples of seed from some of the rutabaga varieties susceptible to clubroot when tested in Wales were found to show little or no infection when grown on clubroot-infested soil in Wisconsin.

In the limited study of inocula from various parts of the United States, including collections from as far east as Massachusetts and as far west as Washington, it appears that they do not differ greatly. It is significant, however, that the Snowball turnip, which remained

healthy throughout many trials with Wisconsin inoculum, was infected in a small percentage of plants when grown on Indiana and Washington inocula and that the American Purple Top rutabaga showed some disease in the New Jersey inoculum. A most exhaustive study of variation of the pathogen within the borders of this country should be carried out. In the meantime it may be assumed that, while cabbage and other members of the kohl group can be expected to be very susceptible to the clubroot organism as it now occurs in the United States, the majority of the varieties of turnip and rutabaga are highly resistant.

SUMMARY

This paper is concerned with a study of the relative resistance and susceptibility to the clubroot organism (*Plasmiodiophora brassicae* Wor.) of the varieties of turnip (*Brassica rapa* L.) and rutabaga (*B. napobrassica* Mill.) in common use in the United States.

Insofar as turnips are concerned, the results are in general accord with those secured elsewhere in that certain varieties are highly resistant, others moderately resistant, and still others very susceptible.

Most rutabaga varieties showed no infection when grown on naturally infested Wisconsin soils, and none showed a high percentage of infection. The fact that some of the varieties used are known to be very susceptible to clubroot in certain European localities indicates a possible variation in pathogenic selectivity within the species *Plasmiodiophora brassicae*.

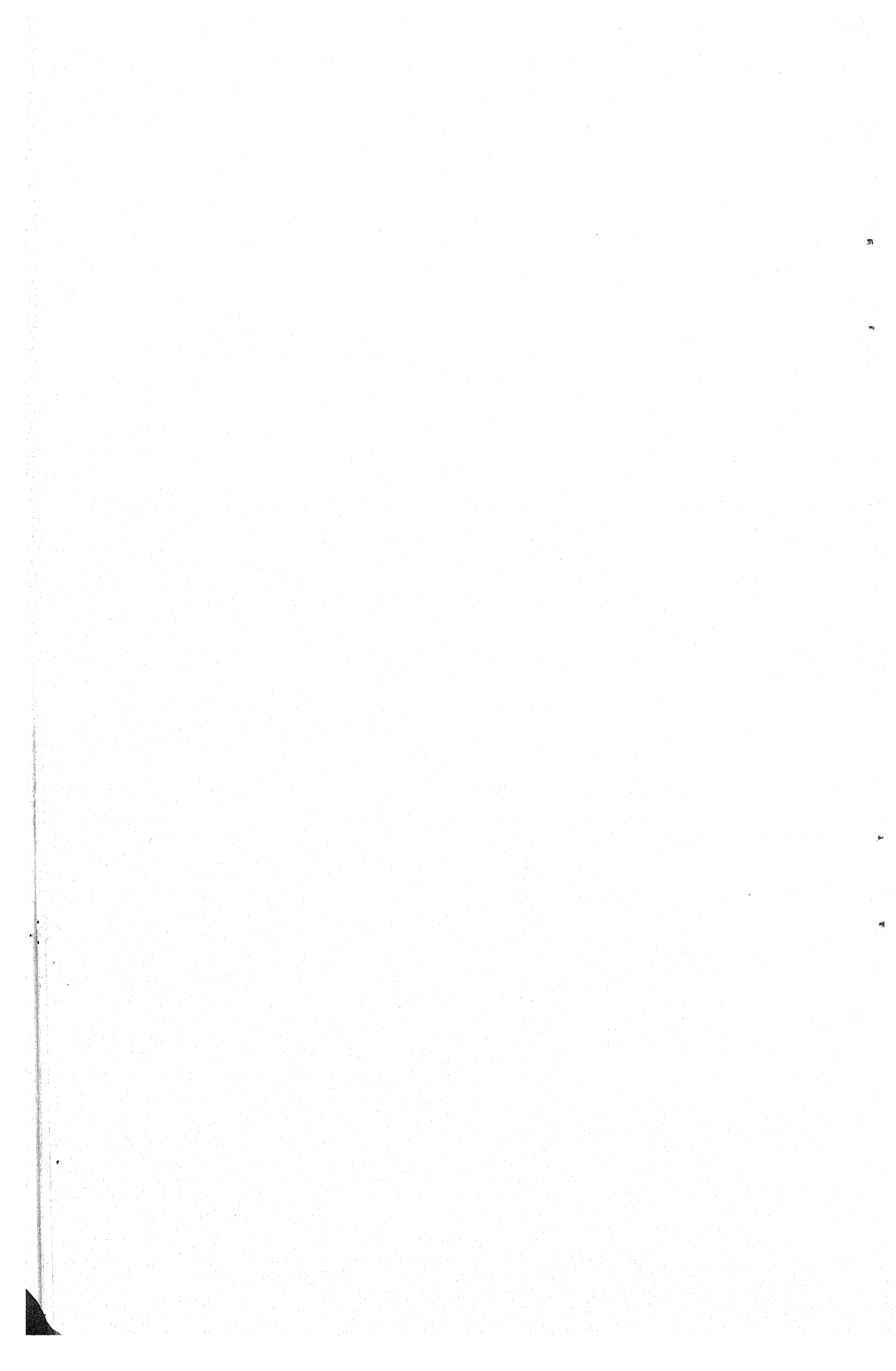
A preliminary study of the pathogenicity of the organism collected from several widely separated localities in the United States indicates very little difference in the pathogenic selectivity of the inocula used. However, low percentages of infection were obtained in some cases on varieties of turnip and rutabaga that had remained completely free in tests on naturally infested Wisconsin soil. The importance of further study of pathogenic variability of the parasite is emphasized.

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TOBACCO FOLLOWING BARE AND NATURAL WEED FALLOW AND PURE STANDS OF CERTAIN WEEDS¹

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INTRODUCTION

In colonial days the growers of tobacco of necessity planted the crop on newly cleared land. It was soon observed that virgin soil produced good yields as well as leaf of high quality well suited to the market demands of the period. Even at that early date the growers of the crop found that after a given field had been used for growing tobacco for a short period the soil became less productive. Consequently the early settlers continually cleared the forested areas in order to consistently produce the desired type of tobacco. It is commonly recognized even today that recently cleared forested land produces a grade of leaf having a fine texture and light body that for many purposes commands a relatively high market price. In recent years, however, the wooded areas have become so limited that it is necessary for growers to produce the major part of the crop on fields that have been cultivated continuously or intermittently for a comparatively long period.

This practice has ultimately resulted in depletion of the available plant-food reserves of the soil so that it has become increasingly necessary to supply soil deficiencies in plant food in the form of manures and fertilizers. It has generally been possible to maintain high yields of tobacco in continuous culture by these practices, provided parasitic diseases do not come in to damage the crop, but it is a common occurrence for the quality of the leaf produced under continuous culture to become unsuited to market demands. In an effort to overcome these difficulties a number of rotation systems in combination with manures and fertilizers have been tried with a fair degree of success. These practices, however, have not proved satisfactory on all soils and with all crop combinations.

The importance of the several plant-food constituents in maintaining yields and values of tobacco has received attention for flue-cured (10)³ and Maryland leaf (8). It is always necessary that these constituents be supplied to the plant in available forms either by the soil or as manures and fertilizers applied to the soil.

The possible contributing factors concerned in the effect of a given crop on the succeeding crop in the rotation have been previously discussed in detail in a paper (5) that gives a review of the literature bearing upon these relationships.

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² Died April 29, 1939.

³ Italic numbers in parentheses refer to Literature Cited, p. 844.

It was early recognized (1891) that weed plants occurring as adventitious vegetation along waysides and elsewhere could be used in preparing composts, since they are relatively rich in plant-food constituents (9). It was pointed out that the different species varied in composition, as shown by published analyses. Additional data giving analyses of weeds under North Dakota conditions also have been published (6). More recently Eisenmenger (2, 3) has conducted studies on the organic decomposition products resulting when plants belonging to various species are incorporated with the soil.

The culture of tobacco in Maryland is unique in that nowhere else in the United States has the crop been grown continuously in a given area for so long a period, about 300 years. This section has furnished the industry with the parent stock for varieties from which many of our important commercial types have developed (4). In addition, it has furnished the testing ground for developing practical methods of maintaining yields and quality of tobacco when grown on a given soil for a long period. Killebrew (7) in 1880 pointed out that yields could be maintained by the use of manures and fertilizers but that the quality of the product was often unsatisfactory. Further observations in many of the older tobacco-producing States were also presented in regard to the maintenance of yields and quality of tobacco on newly cleared lands and in fields that were allowed to lie idle for a period of years. Patterson (11) reported in 1900 that Maryland soils which had lost their capacity to produce high-quality tobacco as a result of continued use in growing this crop could be renovated by allowing them to revert to broomsedge and old-field pine. It has been observed repeatedly, in connection with various experiments conducted over a period of years at the Upper Marlboro tobacco field station, that on cultivating land which had been occupied for a time by native vegetation, consisting of broomsedge or other weeds, tobacco of exceptionally fine quality was obtained for the first and second years following this type of vegetation cover. The crop produced under these conditions more nearly approached that obtained on newly cleared forested areas than under any other set of conditions yet found.

The cause of failure to obtain the type of leaf growth desired in continuous culture is not clear. The exhaustion of plant nutrients undoubtedly is often an important factor, as well as the building up of parasite populations that injure or destroy the growing plant. The development of soil toxins or other substances or conditions deleterious to growth of tobacco appears sometimes to enter the complex, but direct proof or identification of such conditions has not been developed. Apparently the physical condition of the soil induced by, or associated with, the development of adventitious vegetation is a dominant factor in the favorable effects obtained. The mulch produced by the accumulated debris associated with natural weed fallow, by rendering available potassium or other plant foods from the soil minerals (12), may be a factor in the favorable effects reported. This mulch also prevents wind and water erosion, which transports considerable topsoil under some conditions.

In an earlier publication (1) it was pointed out that it has been possible consistently to produce high-quality leaf under Maryland conditions when the crop follows a natural weed fallow of sufficient duration.

PROCEDURE

Since it had been observed that high-quality tobacco (*Nicotiana tabacum* L.) was consistently produced for the first year or two after a growth of natural weed cover, it seemed desirable to study the subject experimentally. Accordingly, in 1922 experiments were undertaken with tobacco grown after natural weed fallow on plots at the tobacco field station, Upper Marlboro, Md. These plots immediately adjoined those employed in tests with legumes and other cover crops, the results of which have been reported elsewhere (1, 5). The plots as originally designed were one-eighth of an acre in size (36 by 151¼ feet). The tobacco plants were transplanted in rows 36 inches apart and 34 inches apart in the drill. There were two series, in one of which tobacco was grown in a 2-year rotation with weeds. After removal of the tobacco crop in early fall the land was left undisturbed about 20 months until it was plowed for the next tobacco crop in the spring of the second year. In another series the plots were occupied by weeds for an additional year. The period between the successive crops of tobacco in this instance was about 32 months. The soil was undisturbed during these periods, since any disturbance would have altered the natural vegetation cover.

These plots were subdivided crosswise in 1925, one-half of each plot being kept free of weeds by frequent hoeing during the summer months. This procedure was adopted in order to determine whether the bare fallow was as effective in maintaining yields and values as the weed fallow. The tobacco on these plots was fertilized with a 1-7-5 mixture applied at the rate of 1,000 pounds per acre during the period 1925-33 and with a 2-8-12 mixture applied at the rate of 750 pounds per acre beginning with 1934. These mixtures were prepared from nitrate of soda, superphosphate, and high-grade sulfate of potash.

Since tobacco following the natural weed fallow made up of adventitious vegetation consistently gave good yields and values, it was deemed desirable to determine whether any of the several species occurring in the weed complex were more effective than the others. In 1931 tests were inaugurated to determine the effects of some of the more common species on the yields and quality of the tobacco crop when grown in substantially pure stands. The plants growing in the natural weed fallow varied to some extent from season to season, apparently depending upon a variety of conditions, more particularly the weather prevailing during the seed-germination period. There was always a more or less dense cover of vegetation throughout the summer months. In the first year, summer annuals predominated, and there was considerable mixture of legumes and nonlegumes. In the second year, some of these annuals reseeded themselves and in some instances wild grasses, including small broomsedge (*Andropogon virginicus* L.) were observed, but the period was too short for establishment of this old-field weed. The trumpet creeper (*Tecoma radicans* (L.) Juss.) frequently came in to a considerable extent the second year from old roots remaining in the soil. Frequently in the first year evening-primrose (*Oenothera*) species were prevalent during the spring.

The weed species included in the pure-culture test were annual sweetclover, Hubam strain (*Melilotus alba* Desr.), rabbitfoot clover (*Trifolium arvense* L.), common ragweed (*Ambrosia artemisiifolia* L.),

wild pea (*Strophostyles helvola* (L.) Britton), partridge-pea (*Cassia chamaecrista* L.), horseweed (*Erigeron canadensis* L.), lambsquarters (*Chenopodium album* L.), and annual lespedeza, Kobe strain (*Lespedeza striata* (Thunb.) Hook. and Arn.). Any of these species may occur in the natural weed fallow to a greater or less extent. However, rabbitfoot clover, wild pea, partridge-pea, common ragweed, and horseweed are the species ordinarily found. It was soon observed that seed of rabbitfoot clover and horseweed commonly germinated in the fall. These species, therefore, passed the winter in the seedling stage, whereas wild pea, lambsquarters, partridge-pea, and common ragweed came up in the spring.

The question at once arose as to the procedure necessary to grow these species in pure stands. There was little or no information available in regard to growth habits or requirements of most of the above-mentioned plants. Since they are generally regarded as objectionable, the only information available was concerned with eradication. However, it may be mentioned here that rarely are any of these species troublesome weeds in cultivated crops. In the earlier years of the tests the young plants obtained from old fields were transplanted in rows 1 foot apart, except wild pea, lespedeza, and sweetclover, which were grown from seed. It has been found possible in recent years to grow practically all of these plants from seed. The procedure followed in seeding ragweed, horseweed, and lambsquarters has been to scatter seed heads over the plots on which they are to be grown after the land has been prepared by a light harrowing. The weed crop was generally unfertilized and grew 1 year, followed by tobacco the second year. The tobacco at the Marlboro location was fertilized with a 2-8-12 mixture applied at the rate of 600 pounds per acre. The fertilizer materials used in compounding this mixture were nitrate of soda, dried blood (one-half the nitrogen from each), superphosphate, and high-grade sulfate of potash.

One series consisted of individual plots, not duplicated, of some of these weed species and a control bare-fallow plot. (See table 4.) The plots of this series were one-hundredth of an acre ($10\frac{1}{2}$ by $41\frac{1}{2}$ feet), with a 2-foot space between plots. Plantings of tobacco were made in rows $3\frac{1}{2}$ feet apart on each of the treatments. The plants were set 29 inches apart in the row. The tobacco was grown in a 2-year rotation with the species shown. The weed growth on these plots was turned under just prior to or immediately after the first killing frost in the fall.

Another series of tests was carried out, consisting in most instances of duplicate plots (see table 5), with all of the above-mentioned species and, in addition, a bare-fallow and a natural weed-fallow plot. The plots in this series were one-seventieth of an acre ($10\frac{1}{2}$ by $59\frac{1}{2}$ feet). Plantings of tobacco were made in three rows to the treatment, $3\frac{1}{2}$ feet apart, with plants spaced at a distance of 29 inches in the drill. A space of 2 feet was provided between plots to prevent cross feeding. The weed growth in these plots was mashed down in the fall and covered with fine-mesh woven wire to prevent the wind from blowing the material from the small areas involved. As a rule the land was plowed for tobacco in the following April.

The above-described tests at Upper Marlboro were located on Collington sandy-loam soil.

In 1933 additional tests were begun at the Pee Dee Experiment Station, Florence, S. C., on Marlboro sandy loam. The plots in this series were one-fortieth of an acre (16 by 68 feet). The plots were separated by a 2-foot space to prevent washing and cross feeding. The rows for tobacco were spaced 4 feet apart and the plants were set 2 feet apart in the drill. The weeds (see table 6) were transplanted in 8 rows to the plot, 2 feet apart. The season's weed growth was chopped up with a stalk cutter after the first killing frost and turned during January for the tobacco crop the following summer. The tobacco was fertilized with a 3-8-6 fertilizer at the rate of 1,000 pounds per acre. The weed growth received no treatment. The fertilizer mixture used in this series was prepared from nitrate of soda and cottonseed meal, each supplying one-half of the nitrogen; superphosphate; and sulfate and muriate of potash, two units or one-third of the potash being derived from the muriate.

The methods used in culture and handling of the tobacco crops grown in these tests will not be considered in detail, but it should be stated that these methods conformed to the best generally accepted local practices. Every effort was made to give each treatment uniform methods of culture and handling. For example, the transplanting was made across the several plots of each series instead of in the direction of a given treatment, so as to equalize plants as to size, freedom from disease, and vigor. Representative samples were selected from each grade of the several treatments after they were stripped, graded, and weighed. The weights thus obtained were used in making calculations of yields. These samples were later submitted to experienced judges of Maryland tobacco, who assigned values. Calculations of gross values per acre were based on these figures.

It was not a simple matter to grow the several wild species of plants in pure culture, since it was necessary frequently to remove by hand any extraneous species appearing in the cultures. Where the cultures were in rows it was possible to remove the undesired kinds with less expenditure of labor. However, in order that the surface of the soil might be covered thoroughly, broadcast seeding was adopted where possible. It has been evident during the later years that many of the weeds have not grown so well as in the earlier years of the test. This has been particularly evident with the partridge-pea.

EXPERIMENTAL RESULTS

Since the weather has a dominant influence on the survival of transplants and the subsequent growth of the tobacco crop, it cannot be neglected in any attempt to arrive at a better understanding of the growth factors operating under field conditions. The rainfall, as recorded at the location of the experiments at Upper Marlboro, Md., and Florence, S. C., arranged in 10- and 11-day periods, is shown in tables 1 and 2. It is practically impossible to summarize the complete records, since frequently short intervals of 1 or more days occurred when the rainfall or temperature was so abnormal as to determine to a large extent the final growth of the crop. It has happened that 5 to 10 inches of rain in a 24- to 48-hour period resulted in almost complete crop failure. Excessive temperature of a few days' duration at times may produce effects from which the crop never completely recovers. The temperature data are not presented for the Maryland location, but should not be greatly different from

those published for Washington, D. C., about 15 miles distant. The temperature data applicable to the South Carolina location are those published by the United States Weather Bureau for Florence, S. C.

TABLE 1.—*Rainfall at Upper Marlboro, Md., for 10- and 11-day periods during the growing seasons, 1925-37*

Month and date	Rainfall during growing season												
	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937
April:	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>
1-10	0.20	1.02	1.83	0.00	0.70	2.80	2.11	0.53	1.98	0.22	3.45	2.11	1.14
11-2066	.26	.07	.93	4.15	.45	.22	2.11	3.90	2.13	.31	.14	.10
21-3080	.33	4.09	3.99	2.34	.00	.66	.63	.00	.49	.77	.18	5.66
Total	1.66	1.61	5.99	4.92	7.19	3.25	2.99	3.27	5.88	2.84	4.53	2.43	6.90
May:													
1-1026	.02	1.92	.29	1.19	.00	2.01	2.04	1.98	1.96	.90	1.32	.79
11-2077	.82	.54	.46	.39	1.60	.66	3.24	1.13	1.88	.57	1.32	1.23
21-31	1.13	.09	.57	1.02	2.56	1.03	1.15	.66	2.43	2.03	1.36	.16	2.21
Total	2.16	.93	3.03	1.77	4.14	2.63	3.82	5.94	5.54	5.87	2.83	2.80	4.23
June:													
1-1000	1.04	.00	.42	.66	.53	3.33	.38	.87	.14	1.91	.18	.61
11-2004	.52	3.25	1.13	3.02	.36	.31	2.11	.66	1.34	1.26	.77	6.31
21-30	1.73	.89	.34	2.28	2.61	.35	.99	.26	.85	.76	.38	1.37	1.47
Total	1.77	2.45	3.59	3.83	6.29	1.24	4.63	2.75	2.38	2.24	3.55	2.32	8.39
July:													
1-10	2.83	1.56	.48	.07	1.99	5.38	5.11	1.07	3.07	.59	2.79	1.01	2.63
11-2032	2.56	.60	2.35	.74	.00	1.71	.97	.50	.07	.80	.17	2.05
21-31	3.55	.65	.33	.00	1.14	1.11	.56	.22	4.78	1.52	.41	2.53	.08
Total	6.70	4.77	1.41	2.42	2.87	6.49	7.38	2.26	8.35	2.18	4.00	3.71	4.76
August:													
1-10	1.12	.54	.07	.89	.90	.00	.76	1.69	.05	.32	.80	.28	.73
11-2085	3.70	2.43	11.72	.00	.12	3.22	.56	1.81	1.30	.37	.08	1.44
21-3165	2.37	3.06	1.15	.45	.04	6.11	.05	8.20	.49	1.08	3.60	5.13
Total	2.62	6.61	5.56	13.76	1.35	.16	10.09	2.30	10.06	2.11	2.25	3.96	7.30
September:													
1-1062	2.48	1.85	2.10	1.33	.22	.43	2.94	.49	4.34	8.00	.10	1.40
11-2092	.0	.44	1.47	1.28	.58	.32	.00	.97	5.29	.08	.15	.26
21-3023	1.61	.00	.74	1.10	.00	1.23	1.17	.68	1.04	.28	2.32	.44
Total	1.77	4.09	2.29	4.31	3.71	.80	1.98	4.11	2.14	10.67	8.36	2.57	2.10
Total for 6 months.....	16.68	20.46	21.87	31.01	25.55	14.57	30.89	20.63	34.35	25.91	25.52	17.79	33.68

TABLE 2.—*Rainfall at Florence, S. C., for 10- and 11-day periods during the growing seasons, 1933-36*

Month and date	Rainfall during growing season for—				Month and date	Rainfall during growing season for—			
	1933	1934	1935	1936		1933	1934	1935	1936
April:	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>	July:	<i>In.</i>	<i>In.</i>	<i>In.</i>	<i>In.</i>
1-10	0.05	0.46	1.12	6.70	1-10	1.01	0.40	1.40	0.35
11-20	1.33	.61	.59	.00	11-20	1.86	2.47	.96	1.10
21-3012	.00	.81	.24	21-31	3.83	1.19	3.13	1.73
Total	1.50	1.07	2.52	6.94	Total	6.70	4.06	5.49	3.18
May:					August:				
1-10	1.47	.00	.30	.05	1-1010	.76	.05	2.74
11-2000	3.64	1.40	.00	11-2088	.35	.91	1.17
21-31	3.24	1.52	.26	.12	21-3104	2.27	3.66	1.21
Total	4.71	5.16	1.96	.17	Total	1.02	3.38	4.62	4.12
June:					September:				
1-10	1.24	1.36	.60	1.54	1-10	2.30	1.62	5.57	1.47
11-20	1.24	.16	.66	4.82	11-2000	1.25	.77	2.88
21-30	1.42	2.30	.00	.51	21-3000	.76	.64	1.93
Total	3.90	3.82	1.26	6.87	Total	2.30	3.63	6.98	6.28
					Total for 6 months.....	20.13	21.12	22.83	27.56

The effects of natural weed fallow and bare fallow on acre yields, gross value, and average price per pound of tobacco (table 3) represent a continuation and extension of previously published data (1). The earlier years (1922-24) are not reported again, since it is desired to point out in the present paper the advantage of weed fallow as compared to bare fallow. The results presented are for the years 1925-37, which is the period in which a direct comparison was made between bare and natural weed fallow. Period averages are presented to show trends under the two systems.

TABLE 3.—*Effects of vegetation cover on the yield, gross value, and average price per pound of leaf tobacco, Upper Marlboro, Md., 1925-37*

[1,000 pounds per acre of 1-7-5 fertilizer used in 1925-33; 750 pounds per acre of 2-8-12 fertilizer in 1934-37]

Treatment	Acre yield									
	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934
2-year rotation:	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
Weed fallow	1,264	1,152	1,504	1,080	1,204	1,064	936	1,224	1,180	1,500
Bare fallow	1,328	1,144	1,374	768	900	776	696	928	688	740
3-year rotation:										
Weed fallow	1,344	1,268	1,488	1,168	1,192	1,080	1,024	1,548	1,064	1,588
Bare fallow	1,412	1,240	1,380	800	916	784	636	948	544	933
Treatment	Acre value									
	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>
2-year rotation:										
Weed fallow	426	415	426	328	503	230	271	317	295	410
Bare fallow	458	389	318	198	276	111	92	82	74	58
3-year rotation:										
Weed fallow	456	441	429	390	431	185	308	418	346	531
Bare fallow	487	435	329	333	243	122	67	96	65	82

Treatment	Acre yield							Average price per pound			
	1935	1936	1937	1925-28	1929-32	1933-37	1925-37	1925-28	1929-32	1933-37	1925-37
2-year rotation:	<i>Lb.</i>	<i>Lb.</i>	<i>Lb.</i>	<i>Lb.</i>	<i>Lb.</i>	<i>Lb.</i>	<i>Lb.</i>	<i>Cents</i>	<i>Cents</i>	<i>Cents</i>	<i>Cents</i>
Weed fallow	1,220	1,098	920	1,250	1,107	1,184	1,180	-----	-----	-----	-----
Bare fallow	784	720	696	1,154	825	726	888	-----	-----	-----	-----
3-year rotation:											
Weed fallow	1,040	1,528	1,009	1,317	1,211	1,246	1,257	-----	-----	-----	-----
Bare fallow	752	888	666	1,208	821	757	915	-----	-----	-----	-----
Treatment	Acre value										
	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>	<i>Dollars</i>				
2-year rotation:											
Weed fallow	347	311	134	399	330	299	339		30	25	29
Bare fallow	111	106	59	341	140	82	179		17	11	20
3-year rotation:											
Weed fallow	270	495	150	429	336	358	373		28	29	30
Bare fallow	121	130	41	396	132	88	196		16	12	21

The data presented in table 4 give yield and value of tobacco following pure cultures of local weeds and following bare fallow. This series differs from that shown in table 5 in that the weeds were plowed under in the fall. The plots were duplicated in most cases in the series shown in table 5. The plot numbers will indicate whether the results shown are averages of duplicate plots or were obtained from single treatments. The weed growth in this series was not plowed under until spring, usually in April.

TABLE 4.—Acre yield, gross value, and average price per pound of leaf tobacco grown in 2-year rotation with pure cultures of the local weeds, Upper Marlboro, Md., 1931-37

[600 pounds per acre of 2-8-12 fertilizer applied to the tobacco crop on all treatments; weeds plowed under in fall]

Treatment	Yield per acre								Average price per pound
	1931	1932	1933	1934	1935	1936	1937	Average 1931-37	
	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds	Cents
Rabbitfoot clover.....	782	1,069	669	1,263	950	938	696	910	-----
Ragweed.....	844	1,276	831	1,175	913	976	875	984	-----
Wild pea.....	932	1,275	1,006	1,513	1,351	1,113	800	1,141	-----
Fallow.....	900	981	757	863	801	764	600	809	-----
Partridge-pea.....	1,100	1,264	931	1,250	1,026	988	651	1,030	-----
Horseweed.....	894	1,269	732	1,163	700	875	513	878	-----
Lespedeza.....	982	1,019	638	1,163	801	901	575	868	-----
	Value per acre								
	Dollars	Dollars	Dollars	Dollars	Dollars	Dollars	Dollars	Dollars	
Rabbitfoot clover.....	176	243	99	249	194	246	44	179	20
Ragweed.....	160	362	143	168	202	282	73	199	20
Wild pea.....	139	322	193	132	237	297	83	205	18
Fallow.....	173	163	59	52	115	157	33	107	13
Partridge-pea.....	154	281	175	242	197	226	47	189	18
Horseweed.....	128	360	144	251	146	241	32	186	21
Lespedeza.....	143	192	77	126	164	251	51	143	16

TABLE 5.—Acre yield, gross value, and average price per pound of leaf tobacco grown in 2-year rotation with pure cultures of the local weeds, Upper Marlboro, Md., 1931-37

[600 pounds per acre of 2-8-12 fertilizer applied to the tobacco crop on all treatments; weeds plowed under in spring]

Plot No.	Treatment	Yield per acre								6-year average price per pound	5-year average price per pound	
		1931	1932	1933	1934	1935	1936	1937	Average			
									1931-37			1932-37
1	Annual sweetclover (Hubam strain).....	Lb. 844	Lb. 1,287	Lb. 1,042	Lb. 1,261	Lb. 1,143	Lb. 1,173	Lb. 901	Lb. 1,093	Lb. 1,135	Ct. -----	Ct. -----
2, 9	Rabbitfoot clover.....	1,003	1,335	1,313	1,025	1,124	1,396	1,007	1,172	1,200	-----	-----
3, 10	Ragweed.....	1,018	1,440	1,309	1,492	1,291	1,322	1,068	1,277	1,320	-----	-----
4, 11	Wild pea.....	1,146	1,453	1,160	1,274	1,105	1,330	924	1,199	1,208	-----	-----
5, 12	Fallow.....	1,059	1,327	1,147	1,020	1,098	1,090	828	1,081	1,085	-----	-----
6, 13	Partridge-pea.....	1,085	1,344	1,287	1,309	1,221	1,146	950	1,191	1,210	-----	-----
7, 14	Horseweed.....	1,189	1,379	1,177	1,235	1,210	1,212	949	1,193	1,194	-----	-----
8	Lambsquarters.....	932	1,286	901	884	459	806	613	840	825	-----	-----
15	Lespedeza.....	1,068	1,208	1,269	963	1,090	1,174	954	1,104	1,110	-----	-----
16	Natural weed fallow.....	1,243	1,086	1,086	1,217	1,204	1,164	929	1,141	1,141	-----	-----
Value per acre												
1	Annual sweetclover (Hubam strain).....	Dol. 193	Dol. 284	Dol. 278	Dol. 340	Dol. 264	Dol. 257	Dol. 80	Dol. 242	Dol. 251	22	22
2, 9	Rabbitfoot clover.....	156	320	314	140	257	382	107	239	253	20	21
3, 10	Ragweed.....	161	434	409	407	375	424	143	336	365	26	28
4, 11	Wild pea.....	179	352	286	171	233	353	123	242	253	20	21
5, 12	Fallow.....	203	291	223	186	158	274	82	202	202	19	19
6, 13	Partridge-pea.....	222	334	350	332	265	260	119	269	277	23	23
7, 14	Horseweed.....	284	447	278	295	312	344	106	295	297	25	25
8	Lambsquarters.....	214	307	184	100	49	147	61	152	141	18	17
15	Lespedeza.....	247	219	181	196	179	343	102	210	203	19	18
16	Natural weed fallow.....	-----	392	278	294	298	334	117	-----	286	-----	25

The results presented in table 6 were obtained from tests at the Pee Dee Experiment Station, Florence, S. C. These tests covered only a

short period of time (1933-36) but were located on a soil differing from that upon which the other tests were conducted. The flue-cured type of tobacco was grown at Florence, and this type differs from that produced in the other tests, which is known as Maryland leaf.

TABLE 6.—Acre yield, gross value, and average price per pound of leaf tobacco grown in a 2-year rotation with pure cultures of some of the local weeds at Florence, S. C., 1933-36

[800 pounds per acre of 3-8-6 fertilizer applied to tobacco on all plots]

Treatment	Yield per acre					Average price per pound
	1933	1934	1935	1936	Average 1933-36	
	Pounds	Pounds	Pounds	Pounds	Pounds	Cents
Ragweed.....	1,758	1,873	1,877	1,330	1,710	-----
Partridge-pea.....	1,254	1,697	1,700	1,240	1,473	-----
Horseweed.....	1,698	1,650	1,653	1,308	1,577	-----
Lambsquarters.....	774	932	927	810	861	-----
Natural weed fallow.....	1,022	1,365	1,408	1,393	1,297	-----
	Value per acre					
	Dollars	Dollars	Dollars	Dollars	Dollars	
Ragweed.....	224	410	413	328	344	20
Partridge-pea.....	100	342	342	337	280	19
Horseweed.....	219	346	351	341	314	20
Lambsquarters.....	72	147	148	226	148	17
Natural weed fallow.....	115	276	297	410	275	21

The percentage of plants harvested from the several treatments shown in table 5 is given in table 7. These data serve to illustrate survival and ultimate growth of tobacco following the various plant species included in the test. The effect of bare and natural weed fallow on size, weight per square foot, percentage of moisture, and fire-holding capacity of leaf tobacco is shown in table 8. The data in this table include physical measurements of properties associated with some of the rather elusive factors of quality. One leaf of average size selected from each of 50 normal plants for the years shown was used to ascertain the size, weight per square foot, and moisture-absorbing capacity when the cured leaf is handled under controlled temperature and humidity. Fire-holding capacity was determined on 50 leaves selected and handled in the same manner.

TABLE 7.—Percentage of the tobacco plants harvested on each treatment when the crop was grown in 2-year rotation with pure cultures of local weeds, Upper Marlboro, Md., 1931-37

Plot No.	Treatment	1931	1932	1933	1934	1935	1936	1937	Average	
									1931-37	1932-37
		Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent	Per-cent
1	Annual sweetclover (Hubam strain).....	100	100	100	92	97	100	100	98	98
2, 9	Rabbitfoot clover.....	97	100	100	91	99	100	97	98	98
3, 10	Ragweed.....	100	100	100	95	100	99	100	99	99
4, 11	Wild pea.....	100	100	91	95	91	100	91	95	95
5, 12	Fallow.....	100	100	100	95	100	100	100	99	99
6, 13	Partridge-pea.....	100	100	100	97	100	100	100	100	100
7, 14	Horseweed.....	100	100	93	100	100	97	100	99	98
8	Lambsquarters.....	100	100	83	71	54	67	76	79	75
15	Lespedeza.....	100	100	100	83	96	79	100	94	93
16	Natural weed fallow.....	-----	100	100	100	100	90	97	-----	98

TABLE 8.—*Effects of bare and natural weed fallow on amount of moisture, size, weight per square foot, and fire-holding capacity of leaf tobacco*

Treatment	Amount of moisture in the leaf, 1935	Average size of leaf				
		1929	1931	1932	1935	Average
		<i>Square feet</i>	<i>Square feet</i>	<i>Square feet</i>	<i>Square feet</i>	<i>Square feet</i>
2-year rotation:	<i>Percent</i>					
Weed fallow.....	21.85	1.19			1.10	1.15
Bare fallow.....	19.43	.75			.67	.71
3-year rotation:						
Weed fallow.....	21.16	.99	1.67	1.49	1.19	1.34
Bare fallow.....	19.75	.92	1.02	.88	.75	.89

Treatment	Weight per square foot					Fire-holding capacity				
	1929	1931	1932	1935	Average	1929	1931	1932	1934	Average
	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Grams</i>	<i>Seconds</i>	<i>Seconds</i>	<i>Seconds</i>	<i>Seconds</i>	<i>Seconds</i>
2-year rotation:										
Weed fallow.....	6.05			5.84	5.95	13.5	47.3	16.9	55.8	33.4
Bare fallow.....	9.22			7.70	8.46	5.4	17.8	12.0	28.9	16.0
3-year rotation:										
Weed fallow.....	6.83	4.27	7.28	5.99	6.09	9.9	50.2	14.3	71.0	36.4
Bare fallow.....	7.90	5.16	8.78	7.26	7.28	6.5	23.0	12.0	22.0	15.9

DISCUSSION

Successful culture of tobacco does not depend simply on the tonnage produced per acre, for the product must meet market demands as to color, aroma, texture, elasticity, body, fire-holding capacity, ability to undergo the aging process with improvement, and other requisites of quality. These diverse and exacting demands can be met only by close attention to details of culture and handling. It is not uncommon for the grower to observe all precautions known to him in regard to these details and yet fail to produce high-quality leaf, owing to the use of undesirable soil, improper cropping systems, inadequate fertilization, the invasion of parasites, and other causes not well understood.

A system that consistently gives good yields of high-quality leaf is always worthy of consideration. It is clearly evident from the data presented in table 3 that high-quality leaf was consistently produced when the tobacco was grown after a natural weed fallow. The crop that followed bare fallow decreased in value until the returns did not defray the cost of production. It is evident that in the earlier years of the test the portions of the plots that were kept bare produced as high crop yields and values as the portions on which the weeds were allowed to grow. The yields and values were maintained under the 3-year system, while the 2-year system showed some falling off though not to any great extent. It is possible that there was an improvement under the 3-year weed rotation, since the top prices paid for Maryland leaf tobacco were much higher during the earlier years of the test than those prevailing in recent years. The yields per acre remained at the same high level of about 1,200 pounds per acre under both the 2- and 3-year systems. The values appear to have fallen off under the 2-year system, since the average of the first 4 years was somewhat higher than that for the last 5 years. The total gross value following the 3-year system of natural weed fallow was

above \$350 per acre for the last 5-year period, which is to be regarded as exceptionally good. Only in the very dry year of 1930 and the very wet years of 1935 and 1937 were the values materially reduced after natural weed fallow. The average price per pound, which serves as a good index of quality, appears to have fallen off in the 2-year system but was maintained at a high level for the tobacco grown in the 3-year system.

The relative merits of some of the wild species commonly occurring in the natural weed fallow as a cover crop for tobacco are well illustrated in table 4. It is evident that all of the species used resulted in increases in yield as compared to bare fallow, when the average for the period is considered. The poorest results obtained in this series were those with lespedeza. The highest gross values per acre were obtained from wild pea, with ragweed a close second. In some seasons the plot with horseweed as the cover crop produced tobacco that showed a high gross value per acre, and the average price per pound from this treatment was slightly higher than from any others. The yields and values obtained in this series, in which the weeds were plowed under in the fall, were lower than where the weed cover was allowed to remain on the soil until spring. It is possible that wind and water erosion was largely responsible for these differences.

Where spring plowing was practiced the highest yields in the series (table 5) were obtained from the ragweed cover. This plot also produced the highest gross value per acre and the highest average price per pound. It is significant that the next highest gross value and average price per pound were obtained from the plot with horseweed as the preceding cover. Natural weed fallow followed horseweed in gross value of tobacco produced and gave the same average price per pound for the tobacco as that obtained from the horseweed plot. In contrast with these results, reduction in yield and value occurred on the plot where lambsquarters was the cover crop (fig. 1), as compared to bare fallow. There also appears to have been no advantage from lespedeza as a cover crop, since the yield and value of the leaf produced was almost the same as on bare fallow. Sweet-clover, rabbitfoot clover, and wild pea did not always show a decided advantage as cover crops to precede tobacco in this series. Partridge-pea appears to have produced some increase in yields and values as compared with bare fallow.

The chief virtue of the weed cover for the succeeding tobacco crop appears to be its effect in inducing a rapid and uniform growth of tobacco from transplanting time to maturity. The tobacco following ragweed (fig. 2) serves as a good example of this effect. In those cases in which the plants do not grow uniformly, as with the lambsquarters (fig. 1) and lespedeza (fig. 3), the stunted plants as a rule show no very definite symptoms under Maryland conditions except those associated with brown root rot. Under South Carolina conditions, when tobacco follows lambsquarters, nematodes often are the most apparent cause for failure of the tobacco plants to grow. It is possible that the inhibitory growth factors not yet identified are the same in both instances and that the two visible causes, brown root rot and nematodes, are only secondary invaders. It has been observed in the case of tobacco following annual lespedeza under Maryland conditions that seemingly the failure to grow uniformly



FIGURE 1.—Tobacco following (A) lambsquarters, and (B) horseweed. Upper Marlboro, Md., August 16, 1935. (See table 5 for yields and values.)

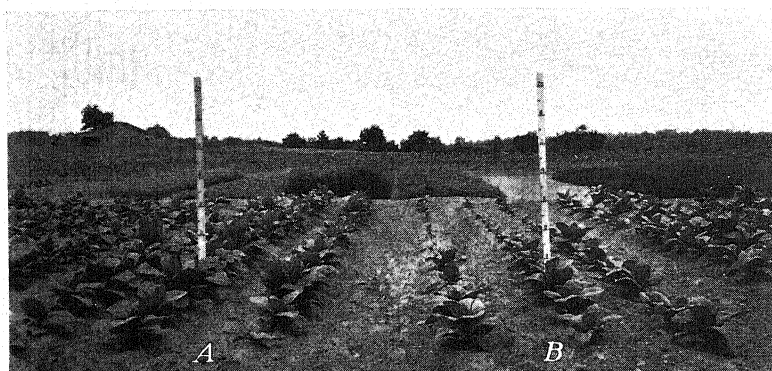


FIGURE 2.—Tobacco following (A) common ragweed, and (B) wild pea. Weeds in background. Upper Marlboro, Md., July 20, 1933. (See table 5 for yields and values.)

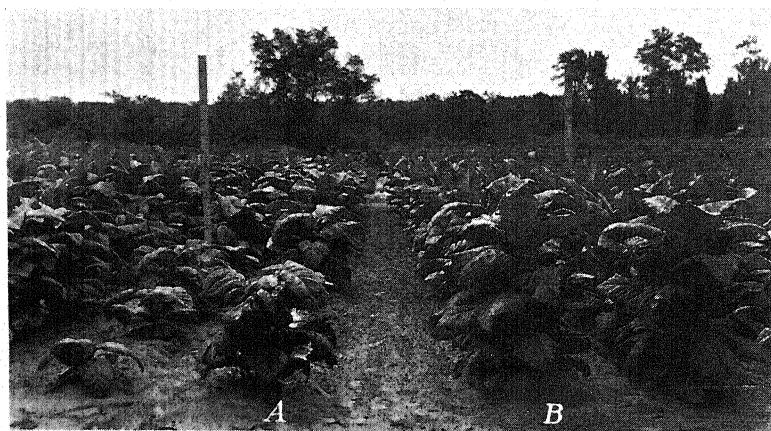


FIGURE 3.—Tobacco following (A) annual lespedeza, and (B) horseweed. Upper Marlboro, Md., September 6, 1934. (See table 5 for yields and values.)

and rapidly is due to brown root rot. Tobacco following lespedeza in some locations frequently shows a heavy invasion of nematodes, while in other locations black root rot (*Thielaviopsis basicola* (B. and Br.) Ferraris) is the most evident cause for failure of the crop to develop normally. Again, the most apparent causes may be only secondary.

While the tests conducted in South Carolina were not so extensive and were not continued for so long a period as those in Maryland, the results secured were nonetheless positive (table 6). Highest yields and values were obtained where ragweed was grown as the preceding cover (fig. 4, *A*). Horseweed gave results that were almost as good as those obtained from ragweed (fig. 4, *B*). The poorest yields and values obtained in the South Carolina tests were from lambsquarters (fig. 4, *C*). Natural weed fallow and partridge-pea, as preceding cover for tobacco, produced, as a rule, high yields and values. Under South Carolina conditions the highest average price per pound was obtained from the natural-weed-fallow combination.

The low yield from lambsquarters was the result, at least in part, of the loss of many plants (table 7) that were stunted in early growth and did not reach sufficient size for harvesting, while the low value can be attributed largely to irregular maturing of the crop because of the stunting. The same situation applies to a greater or less extent to tests with annual lespedeza and some of the other legumes represented in these experiments. The differences in growth following lambsquarters and horseweed are not the result of varying amounts of plant material turned under, since the amount in each case was approximately the same (fig. 5). Differences in the quantity of organic material would appear not to be the explanation for differences in growth of tobacco following the various species.

It is significant that natural weed fallow consisting of adventitious vegetation of 2 years' duration has consistently maintained yields and values of tobacco following this weed cover. The explanation is not evident, but it is clear that this effect is not due simply to allowing the land to lie idle since in bare fallowing the crop has decreased in yield and value. In an effort to learn something as to the factors involved, in 1935 one-half of the bare-fallow plot of the 2-year rotation was covered in the fall (September 25) with weeds harvested from old fields from soils of the Collington series. These weeds were removed in the spring of 1936 prior to preparing the land for tobacco. On one-half of the bare-fallow portion of the 3-year rotation, weeds harvested the fall before and stored under cover until spring were plowed under when the land was prepared for tobacco. The actual quantities used were not determined, but an attempt was made to supply approximately the same quantity of vegetation as was growing on the natural weed-fallow plots. The results are shown in table 9. The data indicate that a vegetation cover over winter seems to be necessary for favorable effects. When plowed in the spring, the soil under the weed fallow and weed mulch over winter was more friable and moist than that on bare-fallow areas. It may be pointed out that where excessive pasturing of livestock is practiced the favorable effects reported from natural weed fallow would not be expected since the field would then tend to approach the bare-fallow conditions.

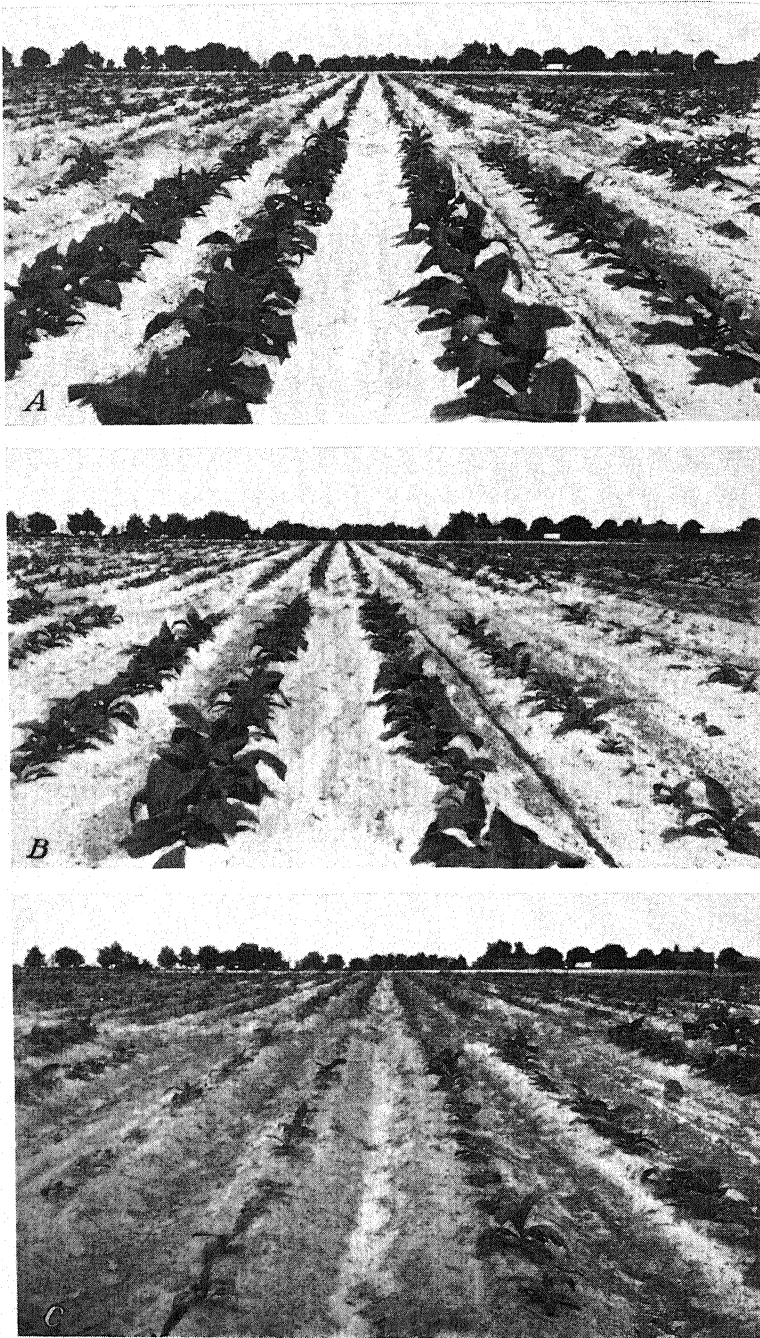


FIGURE 4.—Tobacco following (A) common ragweed, (B) horseweed, and (C) lambsquarters. Pee Dee Experiment Station, Florence, S. C., June 12, 1936. (See table 6 for yields and values.)

TABLE 9.—*Effect of a weed mulch, and of weeds plowed under in the spring, on yield and value per acre of tobacco, Upper Marlboro, Md., 1936*

Treatment	Yield per acre	Value per acre	Treatment	Yield per acre	Value per acre
2-year rotation:	<i>Pounds</i>	<i>Dollars</i>	3-year rotation:	<i>Pounds</i>	<i>Dollars</i>
Bare fallow.....	720	106	Bare fallow.....	888	130
Weed mulch over winter.....	1,080	339	Spring application of weeds.....	760	116
Natural weed fallow.....	1,098	311	Natural weed fallow.....	1,528	495

The tobacco crop may not under all conditions be grown to better advantage after natural weed fallow. In some instances complicating diseases such as bacterial wilt and nematodes are harbored by the prevailing weed growth. The light thin-bodied leaf (table 8) produced as a rule following natural weed fallow, is not suitable for all tobacco-manufacturing purposes. Certain economic relations are



FIGURE 5.—A, Growth of horseweed; B, comparative growth of lambsquarters, Upper Marlboro, Md., August 17, 1931.

to be considered, since in some districts good tobacco soils are scarce and high-priced. It appears, however, that the natural weed-fallow system can be used to advantage where the necessary land is available, a light-bodied leaf is desired, and there are no complicating disease relationships. The beneficial action of the natural weed fallow, especially when combined with intelligent fertilization of the tobacco crop, is reflected in a uniformly high market value per acre and average price per pound, which shows that the product meets current demands for most manufacturing purposes.

A leaf of larger size, with a higher moisture-absorbing capacity and a lighter weight per square foot, is produced on the weed-fallow plot than on the bare-fallow area (table 8). The weight per square foot may be said to represent the so-called body factor, which is so often used in the industry in describing one of the essentials of quality. Weight per square foot varies widely from season to season. This variation appears to be associated with rainfall, since light weight per square foot is definitely related to wet season and more particularly to ample and well-distributed rainfall in July and August.

The fire-holding capacity of leaf tobacco (table 8) is important if it is to be used for smoking purposes. This is particularly true of

Maryland tobacco, since its reputation has been built up largely on its good burning qualities. It is evident that the natural weed fallow has produced leaf tobacco showing a much higher fire-holding capacity than bare fallow, although there is the usual variation from season to season, due, in part at least, to variations in rainfall.

SUMMARY

Since it had been observed that high-quality tobacco was consistently produced for the first year or two after a growth of natural weed cover, experiments were initiated to determine the value of natural weed fallow for preceding tobacco in the rotation as compared with that of certain crop plants and some of the wild species commonly occurring in the natural weed fallow. These comparisons were based on bare fallow as a control.

It is clearly evident from the results herein presented that tobacco which is fertilized intelligently and grown after natural weed fallow of sufficient duration possesses in large measure those desirable characteristics that are observed in the crop grown on virgin land. The crop grown after bare fallow has shown a rapid decline in yield and gross value.

The tests conducted with individual weed and crop-plant species have consistently shown that certain species are much more desirable than others as cover crops to precede tobacco. Tobacco following ragweed and horseweed was markedly superior both in yield and value to that following bare fallow. On the other hand, tobacco following lambsquarters was inferior in yield and value to that following bare fallow. In these tests annual lespedeza has shown no advantage as a cover crop to precede tobacco; sweetclover, rabbitfoot clover, and wild pea have not always shown a decided advantage; while partridge-pea has produced some increase in yield. Although the natural weeds occurring in these tests consisted principally of species that produced high-quality leaf in pure stands, it is possible that those found to be objectionable might predominate, under some conditions, with a resulting harmful effect on the succeeding tobacco crop. It is hardly to be expected that a given weed species would have the same effect on tobacco on all soils or under all conditions.

The generally beneficial effect of the weed fallow was that it promoted a quick start and very rapid and uniform growth of the tobacco plants from transplanting time to maturity. Within normal limits this result is, in turn, associated with a uniformly high market value per acre and average price per pound.

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A STUDY OF HYBRID VIGOR IN A CROSS BETWEEN POLAND CHINA AND DUROC JERSEY SWINE¹

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INTRODUCTION

Cross-breeding has been used for the purpose of recombining the best characteristics of two or more breeds in the formation of a new breed. This is a process that requires many years of continued selection. A second purpose in cross-breeding is to obtain immediate offspring possessing more desirable characteristics, such as greater size, more economical feed utilization, or more rapid growth, than are exhibited by either parental breed.

Interest in cross-breeding for the production of market animals has been of long duration and at the present time is greater in the United States than it has been for decades. The recent success in the production of hybrid corn may have been a stimulating factor in this interest.

The purpose of this experiment was to study the effect of cross-breeding on weight of pigs at birth, vigor, rapidity of growth, and economy of gain.

BREEDS AND METHODS

Duroc Jersey and Poland China breeds were used. Double matings were made according to the following system:

$$\begin{array}{l} \text{Duroc Jersey sow} \times \begin{cases} \text{Duroc Jersey boar} \\ \text{Poland China boar} \end{cases} \\ \text{Poland China sow} \times \begin{cases} \text{Duroc Jersey boar} \\ \text{Poland China boar} \end{cases} \end{array}$$

The two boars were usually mated to the same sow with as little time elapsing between the two services as possible. The same two boars in any one season were mated to all sows. The order of service of the two boars was alternated in order to eliminate the possibility of order of mating affecting the kind of offspring produced.

During gestation the sows were given similar feed and care which were considered to be adequate. After farrowing, the mothers and litters were handled in accordance with the McLean County swine-sanitation system, all receiving similar feed and care.

All pigs were weighed and given individual earmarks at birth, later immunized against hog cholera, and the boar pigs castrated. At weaning or soon thereafter the pigs were separated into the following groups: Purebred Duroc Jersey; purebred Poland China; crossbred pigs from Duroc Jersey female \times Poland China male; and crossbred pigs from Poland China female \times Duroc Jersey male.

The various groups of pigs were fed, in dry lots, shelled corn and a protein supplemental mixture in separate compartments of self-feeders. The supplemental mixture consisted of 2 parts tankage, 1

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part linseed meal, and 1 part alfalfa meal. The pigs were kept on this ration until they reached an approximate weight of 200 pounds.

Weights of pigs were taken at birth, when put into lots, and every 2 weeks thereafter until final weight was reached. Records of feed consumption by groups were also kept.

TABLE 1.—*Relation of time elapsing between services in double-mated sows and parentage of litters*

Interval between services (minutes)	Matings	Litters sired by—			Interval between services (minutes)	Matings	Litters sired by—		
		First boar only	Second boar only	Both boars			First boar only	Second boar only	Both boars
1	Number	Number	Number	Number	15	Number	Number	Number	Number
2	12	3	1	8	18	1	0	1	0
3	22	1	5	16	20	1	0	0	1
4	9	3	0	6	25	1	0	0	1
5	10	2	2	6	240	1	0	1	0
6	12	3	2	7	480	1	1	0	0
7	5	2	0	3	540	1	0	1	0
8	1	0	1	0	720	1	0	1	0
9	2	2	0	0	960	1	1	0	0
10	3	1	1	1					
12	1	0	0	1					
					Total	86	19	17	50

EFFECT OF TIME BETWEEN SERVICES ON PARENTAGE OF YOUNG

The time elapsing between services by the two boars varied from 1 minute to 16 hours, though the majority of double matings were made within a 6-minute interval (table 1). The litter produced in the case of the 16-hour interval was sired entirely by the first boar. The next longest time elapsing between services, was 12 hours, in which case the pigs were from the second boar. The single litters² were sired with approximately equal frequency by the two boars regardless of the time interval, at least up to 12 hours. Nor does the interval between services appear to affect the parentage of pigs in mixed litters.² However, a greater proportion of mixed litters resulted from matings with short intervals between services than from those in which the intervals were longer. Among 65 double matings with intervals between services of 5 minutes or less 43 mixed litters were produced. Among 15 litters with intervals of 6 to 20 minutes inclusive between services only 7 were mixed. No mixed litters were produced in 6 cases in which the interval between services was longer than 20 minutes.

ORDER OF MATING IN RELATION TO PARENTAGE OF PIGS

The order of mating within the time elapsing between services (table 1) had no significant relation to the parentage of the pigs produced. Among 40 litters with only 1 sire represented in a litter,³ the boars used first produced 21 and the boars used last produced 19

² The three kinds of litters produced from double matings are designated as follows: Purebred litter, a litter all pigs of which were sired by the boar of the same breed as that of the sow; crossbred litter, a litter all pigs of which were sired by the boar of the breed different from that of the sow; mixed litter, a litter containing pigs sired by both boars; single litter, a litter all pigs of which were sired by one boar, and may be either purebred or crossbred.

³ In table 1 only 36 litters sired by one boar are shown because the time between services was not recorded for the remaining matings.

litters. However, there was a greater number of litters with all pigs either purebred or crossbred than would be expected on the basis of random fertilization with two kinds of spermatozoa present in equal numbers. The proportions among 105 litters where double mating was used were 24 purebred, 16 crossbred, and 65 mixed litters.

Two possible explanations of this unexpected distribution are: (1) The spermatozoa of the two boars were deposited in different positions in the reproductive tract. Those deposited closer to the oviduct, and consequently to the eggs, would have the first opportunity of fertilizing the eggs. (2) Variation in the relative fertility of the two boars at different services.

While the kinds of litters were not influenced by the order of mating, the kinds of pigs in mixed litters were affected by the order of mating (table 2). In 65 mixed litters containing 637 pigs, 359 were sired by the boar used first and 278 by the boar used last. Assuming numbers of sperm and other conditions to be similar, with random fertilization a ratio of 1:1 in respect to the offspring of the two would be expected. The observed results deviate 40.5 from the expected. The probable

error of this ratio is 8.5. $\frac{D}{PE} = 4.8$, and indicates a significant departure from the expected. With all litters, both single and mixed, the boars used first sired 546 pigs and those used last sired 402.

Among 637 pigs in the 65 mixed litters, 329 were purebred and 308 were crossbred. The deviation here is 10.5 and $\frac{D}{PE} = 1.2$, indicating a close fit to the theoretical expectation. In the matings to produce these 65 litters, boars which would produce purebred pigs were used first 35 times and boars which would produce crossbred pigs were used first 30 times. Since according to these figures, the boars used first have a greater chance of being parents than the boars used last, a correction can be made. On the basis of the foregoing results the first boar has a chance of 359/637 of being the sire of a given pig. Correcting for the five extra matings of boars which would produce purebreds, the ratio becomes 325.8 : 311.2. $\frac{D}{PE} = 0.86$, indicating a very close fit to the theoretical expectation.

TABLE 2.—Number of litters and pigs produced by the first and the second boar

Matings			Single litters sired by—				Mixed litters sired by both boars			
			First boar		Second boar		Lit- ters	Pigs from—		
			Lit- ters	Pigs	Lit- ters	Pigs		First boar	Sec- ond boar	
First boar	Second boar	Sow								
Duroc Jersey	Poland China	Duroc Jersey	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	
Poland China	Duroc Jersey	do	2	16	5	35	15	104	72	
Do	do	Poland China	5	53	4	20	14	77	56	
Duroc Jersey	Poland China	do	10	95	2	7	20	101	82	
			4	23	8	62	16	77	68	
Total			21	187	19	124	65	359	278	

AGE OF BOAR AND NUMBER OF PIGS PRODUCED

Thirteen mixed litters with 125 pigs were produced by double matings in which the boars used first were at least $1\frac{1}{2}$ years older than the boars used last. The first boar sired 62 pigs and the second 73. Sixteen mixed litters with 170 pigs were produced in which the boars used first were at least $1\frac{1}{2}$ years younger than those used last. In this case the first boars sired 103 pigs and the second 67. In mixed litters from boars of the same age (± 3 months) 195 pigs were sired by the first boar and 138 by the second. These figures indicate that younger boars tend to produce more pigs in mixed litters than do older boars but, other things being equal, when two boars are used in double matings more pigs are likely to be sired by the first boar than by the second.

SIZE OF SINGLE LITTERS AS COMPARED WITH MIXED LITTERS

The mean litter size of 40 single litters from double matings was $7.78 \pm .36$, whereas in 65 mixed litters with two sires the litter size was $9.82 \pm .24$, a significant difference. $D = 2.04 \pm .43$. The age of the dam, however, influences the size of the litter. The average age of the mothers of the 40 single litters was 1.88 years, and that of the mothers of the 65 mixed litters, 2.14 years. In order to eliminate the effect of age, 40 mixed litters were taken whose mothers were of the same ages as the mothers of the single litters. The results were still significantly in favor of the litters from two males being larger than litters from single males. With the ages the same, the average size of litters with two sires was $9.70 \pm .26$. The difference between this and

the size of single litters is $1.92 \pm .44$. $\frac{D}{P E} = 4.36$. The exact cause of this

difference is not evident. That it is not due to a higher intrauterine survival of crossbred pigs is indicated by the fact that the 24 purebred single litters had an average litter size of 8.0, while 16 crossbred litters had an average size of 7.4. In these averages, age of sows was not considered, but when only sows of the same ages are used in the computations the average litter sizes for purebreds and crossbreds are 7.4 and 7.0, respectively.

BIRTH WEIGHTS OF PUREBRED AND CROSSBRED PIGS

Birth weights of purebred and crossbred pigs were analyzed in three different ways, taking:

- (1) All pigs whether born in single or mixed litters.
- (2) The average weight of purebred and of crossbred pigs of the same sex in mixed litters. The average weight of purebreds in a mixed litter was paired with the average weight of the crossbreds and analyzed by Student's method.
- (3) Pairs consisting of a purebred and a crossbred pig of the same sex taken at random from each mixed litter and analyzed by Student's method.

By the first method in which all purebred pigs were compared with all crossbred pigs, no significant difference between purebreds and crossbreds was found. The mean birth weight of purebred was $2.62 \pm .02$ pounds and that of the crossbreds was $2.64 \pm .02$.

When the average birth weights of purebred and crossbred pigs of the same sex and litter were compared the only significant difference found was between purebred and crossbred females from Poland China

sows \times Poland China and Duroc Jersey boars. In this case the average weight of the purebreds was $2.61 \pm .05$ and of the crossbreds $2.90 \pm .06$ (table 3). For all purebreds and crossbreds in these mixed litters, the average birth weight of the purebreds is $2.62 \pm .03$ and for the crossbreds $2.72 \pm .03$. The difference is $0.10 \pm .04$, which is not significant.

The third method of analysis was to take pairs, one member of a pair purebred and the other a crossbred of the same sex from the same litter. These pairs were taken at random and the total number was 184. The mean difference is 0.1299 pound in favor of the crossbreds and the value of P is 0.9793. The odds are 194 to 1 in favor of the crossbreds. The average birth weights in pounds of the purebreds and crossbreds in these pairs are 2.63 and 2.76, respectively.

TABLE 3.—Comparison of average birth weights of purebred and crossbred pigs in mixed litters by parentage and sex

Mating			Males			Females		
First boar	Second boar	Sow	Pairs of averages	Purebred	Crossbred	Pairs of averages	Purebred	Crossbred
Duroc - Jersey.	Poland China.	Duroc-Jersey..	Number 22	Pounds $2.55 \pm .084$	Pounds $2.74 \pm .060$	Number 22	Pounds $2.56 \pm .070$	Pounds $2.55 \pm .052$
Do.....	do.....	Poland China.	29	$2.73 \pm .065$	$2.68 \pm .073$	28	$2.61 \pm .052$	$2.90 \pm .060$

STRENGTH OF PIGS AT BIRTH

At farrowing the pigs were classified in respect to strength as strong, medium, weak, and dead except for those in seven litters early in the experiment. On the basis of the appearance and activity of the pigs at birth 3.7 percent more of the crossbreds than of the purebreds were graded strong and also 2.4 percent less of the crossbreds were in the medium class (table 4). The percentage of weak pigs was slightly higher for purebreds, though the percentage of purebreds farrowed dead was lower.

MORTALITY BEFORE VACCINATION

The average age at which the pigs were vaccinated was 38 days. Owing to the presence of some undiagnosed disease in the herd, mortality was exceptionally high one year. In mixed litters (table 5) the mortality of the crossbred pigs was 39.6 percent while that of the purebreds was 48.6 percent. In single litters the mortality was greater in the crossbreds than in the purebreds by 10.7 percent. When all purebred and crossbred pigs are considered, the mortalities in percent are 43.3 and 41.1, respectively.

RESULTS OF FEEDING TESTS

Purebred and crossbred pigs from different dams were not included in the feeding trials, except in one instance, because environmental differences among such pigs are greater than among purebred and crossbred pigs farrowed in the same litter. Such environmental

differences might mask the real effects of cross-breeding. Feeding tests were conducted with only 3 of the 5 crops of pigs. While there were 65 mixed litters at weaning time, the number for 2 years, containing both purebred and crossbred pigs, was considered too small for reliable feeding tests. For this reason only 20 mixed litters were available for the feeding trials.

TABLE 4.—*Strength at birth of purebred and crossbred pigs farrowed in mixed litters*

Condition of new-born animal	Purebred		Crossbred	
	Number	Percent	Number	Percent
Strong.....	195	68.5	200	72.2
Medium.....	40	14.0	32	11.6
Weak.....	36	12.6	28	10.1
Dead.....	14	4.9	17	6.1
Total.....	285	100.0	277	100.0

TABLE 5.—*Number and percentage of pigs alive at vaccination at an average age of 33 days*

Born in—	Pigs farrowed		Pigs alive at vaccination			
	Purebred	Crossbred	Purebred		Crossbred	
			Number	Percent	Number	Percent
Mixed litters.....	329	308	169	51.4	186	60.4
Single litters.....	193	118	127	65.8	65	55.1
Total.....	522	426	296	56.7	251	58.9

Initial weights, average daily gains, and final weights obtained from the feeding trials may be analyzed in various ways. The averages of these measurements for purebreds and crossbreds may be compared directly by including all purebreds and crossbreds used in the experiment (tables 6 and 7). A more critical analysis may be made by comparing the average of the purebreds with that of the crossbreds in each litter. By this method many environmental factors which might otherwise disturb the results are eliminated. The members of the pair have the same mother and have been subjected to the same maternal conditions, which are known to be important. They are of the same age when put into the feed lots. Table 8 gives the results of the statistical analysis of pairs of averages from 20 litters. These pairs were analyzed by Student's method.

The average initial weight of the purebred pigs from the 20 mixed litters as the pigs were started on the feeding trial portion of the experiment was 65.3 pounds. The average weight of the crossbred pigs of the same litters at the same time was 68.4 pounds. The difference is not significant.

The daily rates of gain for averages of purebreds and crossbreds were 1.59 and 1.65 pounds, respectively, again not significantly different.

While on the feeding tests the animals were in groups according to parentage and, therefore, the individual feed consumption is not available. The feed consumption by lots of purebreds and crossbreds is given in tables 6 and 7. The amount of feed consumed per unit of gain in two of the three feeding trials was in favor of the crossbreds

and one was in favor of the purebreds. If all purebreds are compared with all crossbreds the feed consumption per 100 pounds of gain was 409 pounds for the purebreds and 402 for the crossbreds. This is not a significant difference.

TABLE 6.—Weights, gains, and feed consumption of purebred and crossbred pigs for 3 years, 1925, 1927, and 1929

Item	1925				From mixed litters, 1927				From mixed litters, 1929			
	Purebred litter	Purebreds from mixed litters	Crossbreds from mixed litters	Crossbred litter	Purebred Duroc Jerseys	Crossbreds by Duroc Jersey sire	Crossbreds by Poland China sire	Purebred Poland Chinas	Purebred Duroc Jerseys	Crossbreds by Duroc Jersey sire	Crossbreds by Poland China sire	Purebred Poland Chinas
Pigs started.....number	7	5	15	7	8	12	4	18	16	13	14	13
Pigs finished.....do	6	5	14	7	8	11	4	18	15	13	14	13
Average age at start.....days	75	77	78	78	111	107	112	109	130	130	131	132
Average time required to finish.....days	96	102	94	85	98	98	63	84	71	71	77	77
Average initial weight.....pounds	51	39	47	55	60	64	96	72	67	72	67	72
Average final weight.....pounds	204	200	205	205	203	202	208	198	196	200	204	197
Average daily gain.....pounds	1.59	1.57	1.68	1.77	1.45	1.38	1.79	1.50	1.79	1.80	1.78	1.62
Feed consumed per 100 pounds of gain.....pounds	391	401	378	400	423	422	406	427	389	368	411	410

TABLE 7.—Summary of weights, gains, and feed consumption of purebred and crossbred pigs for 3 years, 1925, 1927, and 1929

Item	1925		1927	
	Purebred	Crossbred	Purebred	Crossbred
Pigs.....number	11	21	26	15
Average initial weight.....pounds	47	53	69	70
Average time required to finish.....days	99	91	88	89
Average daily gain.....pounds	1.59±.031	1.71±.018	1.49±.055	1.50±.055
Feed consumed per 100 pounds of gain.....do	396	385	426	418

Items	1929		Totals	
	Purebred	Crossbred	Purebred	Crossbred
Pigs.....number	28	27	65	63
Average initial weight.....pounds	70	70	65	64
Average time required to finish.....days	75	75	84	83
Average daily gain.....pounds	1.71±.022	1.79±.025	1.60±.017	1.69±.026
Feed consumed per 100 pounds of gain.....do	399	405	409	402

TABLE 8.—Initial weights, daily gain, and 6-month weights of purebred and crossbred pigs

[Average of purebreds paired with average of crossbreds in same litter (20 pairs)]

Item	Purebred	Crossbred	Z	Probability
Initial weight.....	Pounds 65.3	Pounds 68.4	0.22	4.4:1
Average daily gain.....	1.59	1.65	.22	4.4:1
Last weight.....	179.1	185.6	.22	4.4:1

Because all pigs of a litter did not finish the feeding test at the same time (they were removed when the individual reached a weight of 200 pounds) their final weights were not taken at a uniform age. In order to compare the average weight of all purebred pigs of a litter with that of all crossbred pigs of the same litter at the same age, it was necessary to select weights which were taken at the same time, but before any of the pigs had been removed from the experiment. The average of the 20-litter averages for purebred pigs was 179.1 pounds and for the crossbreds 185.6. Again the difference is not significant. A summary of these results is given in table 8.

In all comparisons of purebred and crossbred animals in this experiment, including weight at birth, mortality before vaccination, initial weight, rate of gain, feed consumption per 100 pounds gain, and weight at approximately 6 months of age, the only one significantly in favor of the crossbreds is birth weight. The others are in favor of the crossbreds but the difference is in no case significant. One might inquire into the probability of all or several measurements being in favor of the crossbreds when no one is significant. The question, however, is not one of simple probability because of the correlations existing among such things as rate of gain, economy of gain, and initial weights.

The literature on cross-breeding swine is by no means consistent in ascribing beneficial results to cross-breeding. As long as such a condition exists it would seem that a problem of major importance is to learn more concerning the nature of heterosis or hybrid vigor in order that predictability of cross-breeding results might be attained. The system of double mating provides a refinement in experimental technique which this problem in its present state greatly needs.

SUMMARY

Double matings were used with Duroc Jersey and Poland China swine to produce litters that contained both purebred and crossbred pigs.

A significantly larger number of pigs were produced in litters sired by two boars (mixed litters) than in litters sired by a single boar (purebred or crossbred litters).

The birth weights of purebred and crossbred pigs were subjected to three methods of analysis to determine whether the differences between them were significant. By the method considered best adapted to the problem, a small but significant difference in favor of the crossbreds was demonstrated.

Among pigs farrowed alive the strength gradings were slightly in favor of the crossbreds, but a slightly larger percentage of crossbred pigs were farrowed dead.

Mortality before vaccination was slightly less in crossbreds than in purebreds.

Small differences in favor of crossbreds were found in respect to weight at beginning of feeding test, daily rate of gain, feed per 100 pounds of gain, and weight near market age, but these differences were not statistically significant.

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EVIDENCES OF RACIAL INFLUENCE IN A 25-YEAR TEST OF PONDEROSA PINE¹

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INTRODUCTION

The need for discrimination in collecting and importing forest-tree seed for planting was effectively pointed out by European foresters about 50 years ago, and some experimental plantings of seed from different sources were made in Europe at that time. About the opening of the present century, Cieslar (8)² and Engler (12) began intensive systematic investigations of the influence of seed origin on forest trees. The results showed definitely that when seed from the northern part or the higher altitudes of a tree's natural range in Europe was planted in the southern part or at the lower altitudes, the resulting trees grew less rapidly and developed less well than trees grown in the same localities from seed of local origin; and that if the offspring of parents adapted to the long growing seasons of low latitudes and low altitudes were planted in a more severe climate, they failed to survive or were badly deformed by frost injury.

To test the suitability of Scotch pine seed from various sources for planting in given parts of Europe, it was agreed at the 1906 meeting of the International Union of Forest Experiment Stations that the various countries within the range of the species should undertake an intensive cooperative experiment. Seed was collected from 12 widely scattered sources and planted in 1907 and 1908 in typical localities in Germany, Belgium, Netherlands, Sweden, Hungary, Austria, and Russia. In 1908 and 1909 a similar experiment with Scotch pine was started in Switzerland. Summarizations of the 20-year results of the international experiment and the Swiss experiment, by Wiedemann (35) and Burger (5), respectively, confirmed the conclusions derived from the earlier studies and added greatly to knowledge of the subject. They also indicated definite localities the seed of which is suitable for planting in other stated localities in Europe. These experiments and others having to do with the significance of seed origin have given rise to a wealth of literature, the extent of which is indicated by a bibliography compiled by Champion (7) containing 166 titles.

In American forestry literature, attention was called to the importance of seed origin by Zon (36) in 1913 and by Toumey (32) in 1914. Since then Pearson (24), Eckbo (11), Roeser (27), Bates (4), Austin (1), Baldwin (3), and other American foresters have published discussions of the subject. It has been common practice in

¹ Submitted for publication July 20, 1939.

² Italic numbers in parentheses refer to Literature Cited, p. 886.

the United States to collect forest-tree seed wherever it happened to be abundant and easy to obtain, with little regard to suitability for the locality where trees were to be grown or to the growth characteristics, form, or resistance to frost and disease that the trees might inherit. Only recently has some slight attention been given to the subject by planting and seed-collecting agencies.

As early as 1911 Zon, then in charge of silvicultural research in the Forest Service, started progeny experiments patterned after those in Europe at several of the newly established forest experiment stations in the Western States. Of these experiments, in which several species were used, only a few with ponderosa pine (*Pinus ponderosa*) and one with Douglas fir (*Pseudotsuga taxifolia*) (17, 22) are still in progress and are now yielding results.

The experiment with ponderosa pine discussed in this paper³ is one of these early projects and is, therefore, practically contemporaneous with the afore-mentioned European experiments. Seedlings grown from seed collected in many widely separated localities within the natural range of ponderosa pine were planted on an area in northern Idaho. The first plantings were made in the fall of 1911. The observations reported here extended to 1935.

The purposes of the study were stated at the time of its initiation as follows: (1) To determine the suitability of ponderosa pine seed from different sources for planting in northern Idaho; (2) to ascertain heritable characteristics of growth, form, and hardiness, developed through adjustment of parents to local climates; and (3) to determine what limitations should be placed on the interchange of seed between localities of different climate.

Nothing was known of the pollinating parents as distinct from the cone-bearing parents, and no information was recorded as to individual characteristics of the latter. In practical artificial reforestation, however, in which seed must be collected on a large scale and the forester is not yet able to control or identify the source of pollen, this lack of knowledge of individual parent trees will not seriously affect the application of results. Knowledge of the adaptability of trees grown from seed introduced from different localities is in itself a considerable contribution to the improvement of reforestation practice.

The existence of climatic races of forest trees is most evident in species having wide distribution in latitude, longitude, and altitude. Shaw (29, p. 23) states that—

“the range of variation is somewhat proportionate to change of climate. * * * The western species of North America, for instance, are much more variable than the eastern species, while in Mexico, a tropical country with snow-capped mountains, the variation is greatest.”

Thus ponderosa pine is particularly appropriate for a study of racial influence, for it is probably the most widely distributed conifer of western North America and certainly occurs in as great a diversity of climates as any other. Its range, according to Sudworth (30) and Munns (22a), extends in latitude from 23° in Mexico to 51°30' in British Columbia, and in longitude from 98° in Nebraska to 124° in California.

³ It is desired to acknowledge contributions to this project by D. R. Brewster, who prepared the working plan and supervised the installations, J. A. Larsen and G. Kempff, who made early examinations and records, C. A. Wellner and G. M. Fisher, who made recent measurements, and the late L. G. Hornby, who gave valuable help and advice in organizing the current results.

Two broad forms of ponderosa pine have long been recognized by foresters and dendrologists (16, 28, 30⁴)—the *scopulorum* form, occurring mainly east of the Continental Divide, and the *ponderosa* form, occurring west of the Divide. Some authorities have recognized the *scopulorum* form as a variety and some have given it specific status. In the present study such division of the species was disregarded and progenies were classified solely on the basis of evidence produced by the study.

CLIMATIC DATA OF THE LOCALITIES OF SEED ORIGIN

The various localities in which the ponderosa pine seed used in this study was collected are shown in figure 1. The climatic regions indicated were delimited chiefly on the basis of data on precipitation types given by Ward (33). The boundaries of these regions as shown must be regarded as approximations only. The South Pacific region is the only one not represented by any of the seed used. Each seed-source locality has been designated by the name of the national forest that contains it. Of the three localities on the Bitterroot Forest, at altitudes of 4,000, 5,000, and 7,200 feet, each has been treated separately and further designated by its elevation. Weather Bureau records were obtained for the stations nearest to and most representative of the individual localities of seed origin. Table 1 locates each point where seed was collected and gives details of the Weather Bureau stations at which the records were taken, and the periods of years represented by the records.

Mean annual and mean monthly precipitation was determined for each locality. These data are shown graphically in figure 2. Precipitation and temperature records are given in detail in table 2. Because precipitation and temperature in any locality vary with altitude, it was necessary in a few instances to make some adjustment for the considerable differences in altitude between place of seed origin and the nearest weather station. The adjustments made to render the records representative of these seed-collection sites are explained in the footnotes to the table. Table 2 further contains precipitation-effectiveness indices, showing the balance between evaporation and precipitation; the larger the values, the better the net result in plant growth.

Large variations in several factors are conspicuous as between the different localities. The lowest average annual precipitation was 13.05 inches, for Ashley, and the highest was 51.48 inches, for Siskiyou; the lowest July-August precipitation was 0.36 inch, for Siskiyou, and the highest was 8.83 inches, for Santa Fe.

Annual mean temperature was lowest (33.5° F.) for Bitterroot 7,200 feet and highest (50°) for Siskiyou. For January, the coldest month in every locality, Custer had the lowest mean, 16.8°, and Siskiyou the highest, 35°. The highest and lowest temperatures, 114° and -57°, were recorded in the same locality, Custer. The most equable climate is that of Siskiyou, where the extremes were 108° and -2°.

⁴ Also a preliminary study (unpublished) of the western yellow pine made by H. M. Curran in 1905. In Forest Service files.

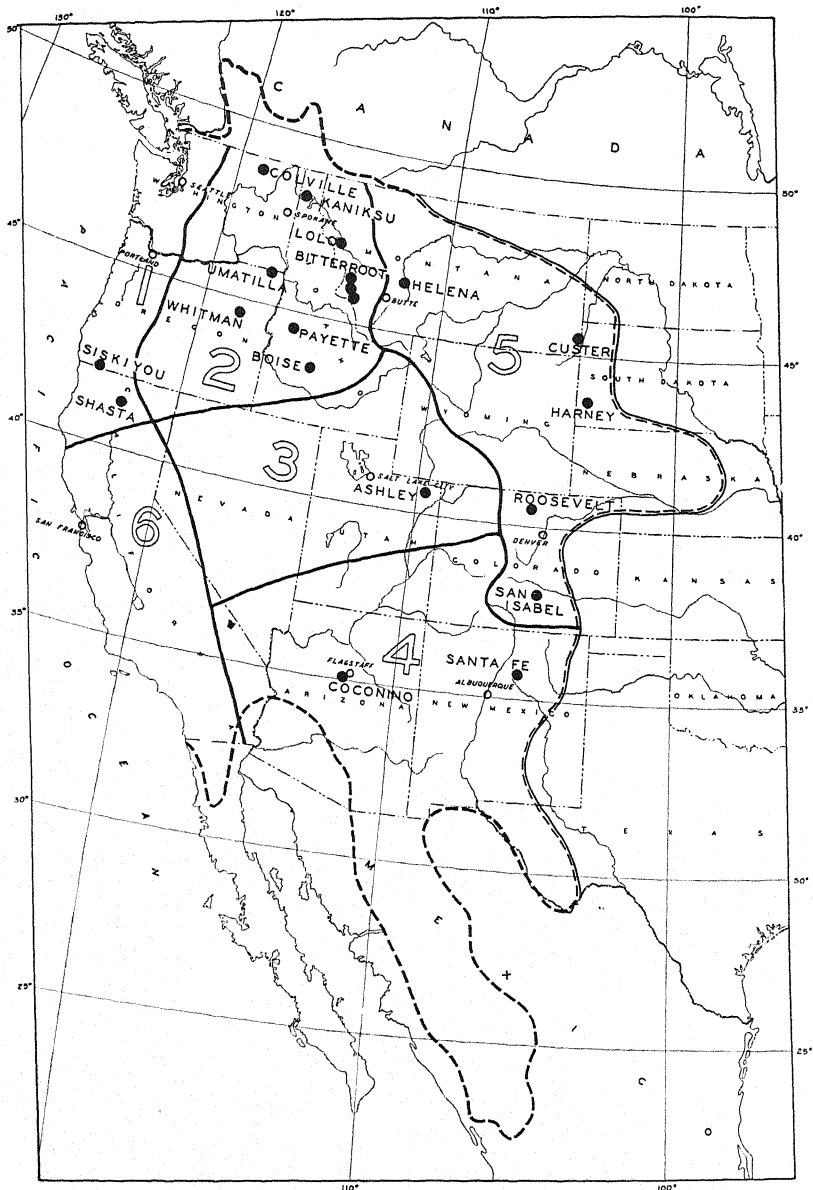


FIGURE 1.—Climatic regions of ponderosa pine range: (1) North Pacific; (2) north plateau; (3) central plateau; (4) south plateau; (5) east of Continental Divide; (6) South Pacific. The broken line shows the range of ponderosa pine according to Sudworth (30) and Munns (22a). Localities from which seed used in experiment was derived are indicated by black dots.

TABLE 1.—*Details of seed-collection points and corresponding weather-recording stations*

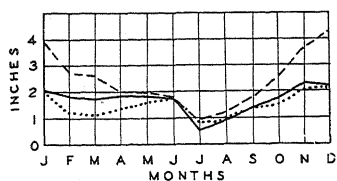
Location of seed-collection point ¹				Locus and extent of weather record				
Region and forest	Latitude	Longitude	Altitude	Locality	Altitude	Distance from seed source	Period of record	Length of record
North Pacific:	° ' "	° ' "	Feet		Feet	Miles		Years
Siskiyou.....	42 05	123 40	2,000	Waldo.....	1,650	5	1915-1935	16
Shasta ²	41 30	122 20	4,000	McCloud.....	3,270	20	1911-1934	24
				Mount Shasta.....	3,555	15	1888-1934	47
North plateau:								
Boise.....	43 30	115 00	5,500	Soldier Creek.....	5,755	20	1910-1935	26
Payette.....	44 30	116 00	5,000	McCall.....	5,025	20	1905-1935	22
Whitman.....	44 38	118 25	5,000	Austin.....	4,200	4	1916-1935	11
Umatilla.....	46 00	117 30	3,500	Wallowa.....	2,935	28	1915-1935	21
Colville.....	48 40	119 00	2,700	Laurier.....	1,644	40	1919-1934	25
Kaniku.....	48 20	116 50	2,600	Republic.....	2,628	10	1900-1934	29
				Priest River Experiment Station.....	2,380	1	1912-1935	24
Lolo.....	47 10	114 50	3,000	Superior.....	2,975	3	1914-1934	19
			4,000					
Bitterroot.....	46 00	114 20	5,000	Como.....	3,750	5	1908-1921	14
			7,200					
South plateau:								
Coconino.....	35 10	111 50	7,100	Williams.....	6,750	20	1888-1934	35
				Flagstaff.....	6,907	20	1888-1934	44
				Fort Valley.....	7,300	25	1909-1934	22
				Roadada.....	7,150	25	1904-1926	23
Santa Fe.....	35 40	105 30	8,000	Gallinas Planting Station.....	7,500	15	1907-1930	24
				Winsor's Ranch.....	8,000	25	1895-1935	40
East of Continental Divide:								
Helena.....	46 30	111 50	4,500	Helena.....	4,110	10	1880-1934	55
Custer.....	45 30	104 00	3,200	Camp Crook.....	3,103	10	1892-1935	42
Harney.....	43 40	103 30	5,000	Deadwood.....	4,535	40	1917-1930	14
				Custer.....	5,309	5	1911-1935	23
Roosevelt.....	40 30	105 40	8,000	Moraine.....	7,775	10	1890-1916	26
				Estes Park.....	8,000	5	1909-1934	26
San Isabel.....	38 00	105 00	8,000	Goodpasture.....	6,120	10	1917-1926	8
Central plateau:								
Ashley.....	40 40	109 40	7,500	Manila.....	6,225	20	1910-1934	25
				Elkhorn-Ashley.....	6,657	10	1910-1934	25
				Fruitland.....	7,000	50	1910-1929	20

¹ The localities are designated in the text by the names of the national forests containing them. The three localities on the Bitterroot National Forest are further designated by elevation. Seed was also collected on the Coeur d'Alene National Forest but proved to be valueless for this experiment since it comprised 2 distinct forms of ponderosa pine.

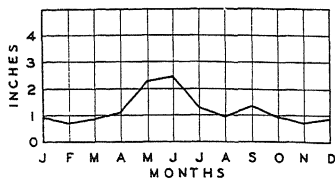
² All trees from this seed source were lost by freezing in 1924.

In considering the precipitation and temperature values in table 2 it should be borne in mind that these factors exert their influence on trees not independently but in combination, and that the altitude of the band on which ponderosa pine occurs increases from north to south, approximately at the rate of 350 feet for each degree of latitude.

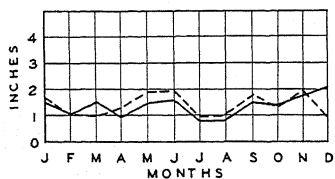
Because of the many factors involved for which no exact data on degree of influence are available, it is evident that only general conclusions can be reached as to correspondence between climate and regional form of ponderosa pine. Furthermore, the number of parent localities represented in this study is too small to make possible a close definition of the range of any regional form.



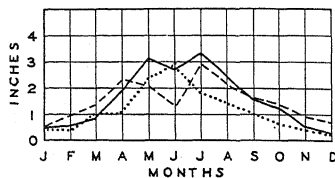
KANIKSU --- 28.70 in. annual
 COLVILLE 17.28 in. annual
 UMATILLA — 20.39 in. annual



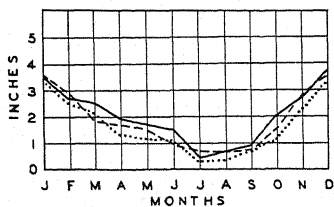
HELENA — 14.27 in. annual



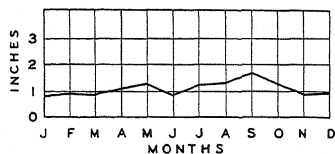
LOLO — 15.95 in. annual
 BITTERROOT --- 16.53 in. annual



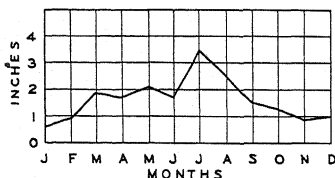
CUSTER 13.89 in. annual
 HARNEY — 18.63 in. annual
 ROOSEVELT --- 17.82 in. annual



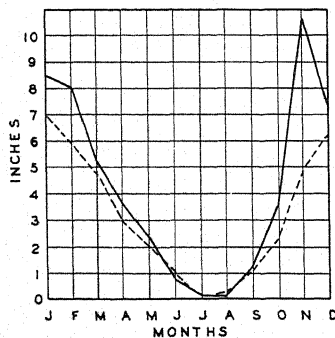
WHITMAN 19.48 in. annual
 PAYETTE — 24.30 in. annual
 BOISE --- 22.21 in. annual



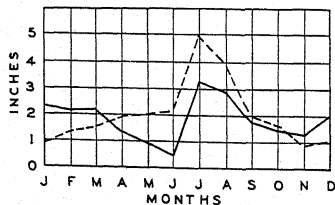
ASHLEY — 13.05 in. annual



SAN ISABEL — 19.80 in. annual



SISKIYOU — 51.48 in. annual
 SHASTA --- 38.47 in. annual



COCONINO — 22.06 in. annual
 SANTA FE --- 24.12 in. annual

FIGURE 2.—Precipitation types of localities of seed origin.

TABLE 2.—Summary of weather records for localities of seed origin

Region and forest	Average precipitation ¹				Mini- mum annual precip- itation	Average temperature ²			Extremes of temperature		Average date of 40° F. daily mean	Precip- itation- effec- tiveness indices ³	Aver- age frost- less season ⁴	Latest frost on record ⁴	Earliest frost on record ⁴
	Annual	April, May, and June	July and August	April- August, and in- clu- sive		Annual	April, May, and June	July and August	Highest	Lowest					
Inches	Inches	Inches	Inches	Inches	° F.	° F.	° F.	° F.	° F.	Days	° F.	Days	June 11	Sept. 3	
North Pacific:														Aug. 24	
Siskiyou.....	51.48	6.49	6.49	6.85	36.79	50.0	54.1	66.6	108	-2	164.1	139	July 6	Aug. 24	
Shasta.....	38.47	5.83	5.83	6.25	21.33	47.0	51.3	63.7	106	-11	138.0	108	July 6	Aug. 24	
North plateau:															
Boise.....	22.21	4.10	1.25	5.35	13.45	42.3	48.8	65.1	100	-26	85.0	78	July 30	Aug. 1	
Payette.....	24.30	5.11	1.03	6.14	13.82	40.2	47.0	62.4	104	-46	95.6	69	July 26	Aug. 4	
Whitman.....	19.48	3.41	.61	4.02	12.61	39.0	46.1	58.7	101	-40	79.5	110	June 30	Aug. 4	
Umatilla.....	20.39	5.44	1.49	6.93	10.60	43.3	50.3	62.9	106	-40	65.4	88	July 14	Aug. 15	
Colville.....	17.28	4.56	1.75	6.31	9.81	43.6	51.7	63.7	109	-35	52.9	103	July 14	Aug. 6	
Kaniksu.....	28.70	5.52	1.87	7.39	16.02	43.3	50.8	63.2	102	-35	164.9	103	July 27	Aug. 13	
Lolo.....	15.95	4.06	1.57	5.63	7.61	44.9	52.1	66.0	104	-33	46.5	103	July 1	Do.	
Bitterroot:															
4,000 feet.....	16.53	5.05	1.95	7.00	13.58	44.2	51.4	64.0	103	-33	45.4	127	June 8	Aug. 25	
5,000 feet.....						41.0	48.2	60.8							
7,200 feet.....						33.5	40.7	53.3							
South plateau:															
Coconino.....	22.06	2.83	6.20	9.03	11.79	45.4	50.7	64.1	102	-33	62.6	110	July 29	Aug. 15	
Santa Fe.....	24.12	6.04	8.83	14.87	14.84	42.9	48.1	59.4	92	-33	39.0	116	July 21	Aug. 20	
East of Continental Divide:															
Helena.....	14.27	5.80	2.10	7.90	7.38	42.2	50.3	65.2	102	-43	36.1	145	June 9	Aug. 25	
Custer.....	13.89	6.30	3.16	9.46	43.3	43.3	53.1	69.1	114	-57	28.5	133	June 20	Aug. 28	
Harney.....	18.63	7.61	5.86	13.41	9.27	41.9	49.4	64.1	100	-31	41.3	112	June 17	Aug. 25	
Roosevelt.....	17.82	5.68	4.90	10.58	11.08	41.4	46.6	59.8	98	-42	48.1	91	July 20	Aug. 17	
San Isabel.....	19.80	5.54	5.97	11.51	13.35	41.7	48.6	62.3	96	-36	20	52.7	July 10	Aug. 17	
Central plateau: Ashley.....	13.05	3.34	2.51	5.85	6.35	39.6	46.4	61.2	94	-36	35.1	85	July 10	Aug. 25	

¹ All the precipitation figures, except those for 4 localities, are exactly as shown by the basic weather-station data used. For the Whitman and Helena localities the weather-station records were increased 10 percent, and for the Umatilla locality they were increased 25 percent, to make them representative of the seed-collection points. This was done on the advice of the local forest officers, and by checking against precipitation maps of the Atlas of American Agriculture (15). For the Coconino locality the local forest officer recommended the use of the Laurier precipitation records as more representative than those of the more closely located Republic weather station. With these corrections, the figures approximate closely the precipitation of 16 of the localities of seed origin. The Ashley, San Isabel, Bitterroot 5,000 feet, and Bitterroot 7,200 feet seed-origin points are 813 to 3,450 feet higher than the local weather stations, and doubts have somewhat greater precipitation than is shown here; but basic data are not available for correction.

² To make temperature figures of weather stations representative for seed-origin localities at greater altitudes, the customary reduction of 3.3° F. was made for every 1,000-foot difference in altitude.

³ Precipitation-effectiveness indices for weather stations, computed by Thornthwaite's (37) method for integrating the effect of evaporation and precipitation.

⁴ The frost figures are those of the weather stations, without adjustment. Except for the Umatilla, Bitterroot, Ashley, and Helena localities, it is believed that the frost conditions at the localities of seed origin are but slightly different from those at the weather stations.

EXPERIMENTAL AREA AND PLOTS

The experimental plantation is a part of the Priest River experimental forest of the Northern Rocky Mountain Forest and Range Experiment Station, located on the Kaniksu National Forest, in northern Idaho. It has an elevation of 2,380 feet. The climate is typical for the western white pine forest, with a mean annual precipitation of 28.70 inches, a moderately deep accumulation of snow, and a fairly heavy spring rainfall. July and August are characteristically hot and dry, with an average total of only 1.87 inches of rain and with prevailingly low relative humidity, which frequently goes below 15 percent. The annual mean temperature is 43.3° F., and frost may occur in any month.

The Kaniksu Forest is mainly occupied by the typical mixed stands of the western white pine type, composed principally of western white pine (*Pinus monticola*), western larch (*Larix occidentalis*), Douglas fir, lowland white fir (*Abies grandis*), western hemlock (*Tsuga heterophylla*), and western red cedar (*Thuja plicata*). Dry south slopes within the type often contain some ponderosa pine in mixture with the less moisture loving of the above species.

The progeny plots are situated in a clearing near the outer edge of a bench otherwise occupied by a natural second-growth stand in which larch and Douglas fir predominate over western white pine. A southwest slope that rises from the edge of the bench close to the plots has a natural open cover of ponderosa pine, larch, and Douglas fir. The bench has the appearance of an old river terrace. Lapham and Youngs⁵ mapped the soil as Springdale sandy loam and described it as follows:

The surface soil of the Springdale sandy loam is a friable fine to medium sandy loam, containing a small amount of gravel * * *. This material extends to a depth of 1 to 2 feet, * * * and overlies a subsoil of loose sand and gravel. This gravel is largely granitic and may be glacial outwash. The substratum, [which is] at a considerable depth, consists of old-lake clays.

* * * The surface drainage is good and the subdrainage is generally excessive. The water-holding capacity is low due to thinness of the soil above the porous subsoil. This porous material also minimizes the upward movement of the capillary moisture. Altogether this is a droughty soil.

Small gravel pits were dug at three widely scattered points on the bench to a depth of 8 feet without exposing the clay substratum. The soil is very deficient in humus as a result of forest fires, which in about 1855 destroyed the virgin forest and some 40 years later destroyed a second-growth stand. A record made in 1917 stated that the soil retained moisture fairly well until about the middle of August, when it dried out to a moisture content of 3 or 4 percent of oven-dry weight at a depth of 12 inches, and then remained unchanged until the beginning of fall rains in September. Soil samples taken at a depth of 24 inches in the centers of all plots on August 31, 1936, had a moisture content ranging from 3 to 9 percent and averaging 4.7 percent. Recent soil-acidity tests made at three points on the planted area showed pH values ranging from 6.2 to 6.8. Rather too well drained for western white pine, the site of the plots appears from the growth rate and vigor of the planted trees to be well suited for ponderosa pine.

⁵ LAPHAM, M. H., and YOUNGS, F. O. SOIL SURVEY OF THE PRIEST RIVER FOREST EXPERIMENT STATION. 1925. [Unpublished manuscript.]

As site conditions are practically uniform on the cleared part of the bench, and as the progeny plots form a small, compact block only 200 by 260 feet in size, the plots may be said to have closely comparable growing conditions. Because the foot of a slope touches the northeast corner of the plantation, however, three plots (the Bitterroot 7,200 feet, the Lolo, and the Bitterroot 4,000 feet) might be assumed to have an advantage as to soil moisture. As the slope has a southwest exposure, however, and the short distance to the ridge top precludes retention of any great amount of water, the advantage if any is not marked. Actual tests showed the Lolo and Bitterroot, 4,000 feet, plots to have slightly less soil moisture than the average for all the plots.

Although the crown canopy on some of the plots has become dense enough to shade out low vegetation in spots, most of the ground surface is still covered with herbaceous and shrubby vegetation of vary-

Payette June 5, 1912	Coconino May 10, 1912	Santa Fe May 3, 1915	Lolo May 2, 1916	Bitterroot 7,200 feet May 29, 1917
Coeur d'Alene Oct. 6, 1911 (excluded)	Custer Oct. 6, 1911	Ashley May 13, 1915	Kaniksu May 2, 1916	Bitterroot 4,000 feet May 31, 1917
Helena Oct. 14, 1911	San Isabel Oct. 14, 1911	Roosevelt June 5, 1912	Bitterroot 5,000 feet May 13, 1915	Siskiyou May 3, 1916
Shasta May 13, 1915 (excluded)	Umatilla Nov. 18, 1911	Whitman May 13, 1916	Boise May 13, 1915	Colville May 13, 1915
				Unknown Origin April 29, 1916 (excluded)

FIGURE 3.—Arrangement of progeny plots, and dates of first planting. The large plots are 50 by 50 feet and the small ones 25 by 50 feet.

ing density. The most abundant species are *Arctostaphylos uva-ursi*, composing 25 to 75 percent of the low vegetation, and *Calamagrostis rubescens*, composing 10 to 65 percent. Other characteristic species, in the order of their abundance, are *Fragaria glauca*, *Pentstemon* sp., *Achillea lanulosa*, *Symphoricarpos racemosus*, *Odostemon aquifolium* (syn. *Berberis aquifolium*), *Pteridium aquilinum pubescens*, and *Ceanothus velutinus*. All these species are typical of the drier sites in this locality.

The progeny trees representing each locality of seed origin were planted on 1 of the 22 plots shown in figure 3. On each of the 18 large plots, 50 feet square, 100 trees were planted, and on each of the 4 small ones, 25 by 50 feet, 50 trees were planted. Spacing of trees was exactly 5 by 5 feet.

The stock used in the first 8 plot installations, made in the fall of 1911 and the spring of 1912, and in 3 made in 1913, 1915, and 1916, was grown in Forest Service nurseries in various regions. For the 11 other installations, made in 1915, 1916, and 1917, stock was grown in a small nursery at the site of the experiment. The trees were planted

on the plots as 2- and 3-year-old transplants. The method was to dig an open hole, spread the roots around a mound of earth in the center, and firm the soil by hand.

For 3 to 5 years after each first installation, all trees that died were replaced with trees of the same lot that had been reserved in the nursery, in order to maintain closed-stand conditions.

Of the 22 progenies shown in figure 3, only 19 are treated in this report. All the trees on the Shasta plot were lost by freezing in 1924. Because its seed-source record is very questionable, the plot designated "Unknown origin" was omitted. The plot designated "Coeur d'Alene" has a mixture of two distinct forms of ponderosa pine and was therefore excluded also.

Every year from 1912 to 1919, the plots were examined and a record was made of the number of living and dead trees, the number of replacements, and the height of a representative 20 percent of the trees on each plot. Similar records including heights of all trees were made in 1927 (14). In 1935, measurements of height and diameter were made on all the trees, and also of internode lengths of the main stem for the years 1930-35. Records were made, also, of survival, vigor, dominance, and foliage characteristics.

FOLIAGE CHARACTERISTICS

The foliage of each progeny was classified as to number of needles per fascicle, length of needles, number of years needles were retained, general appearance of foliage, and internal structure of needles. In this connection it should be kept in mind that the progenies are about the same age, are situated close together on level ground practically uniform as to soil and moisture, and are uniformly exposed to sun and wind.

NUMBER OF NEEDLES IN FASCICLE

In discussing the pines, Shaw (29, p. 4) says:

The number of leaves in the fascicle is virtually constant in most species, the variations being too rare to be worthy of consideration. With some species, however, heteromerous fascicles are normal. The influences that cause this variation are not always apparent (*echinata*, etc.), but with *P. ponderosa*, *leioophylla*, *sinensis*, and others, the number of leaves in the fascicle is, in some degree, dependent on climatic conditions, the smaller number occurring in colder regions.

The fascicles of *Pinus ponderosa*, Shaw states, consist prevailingly of three needles each, but range from two to five or more, the larger numbers occurring in the southern part of the tree's range.

In the progenies of this experiment the number of needles to the fascicle varied from two to three. To determine the proportions of two- and three-needle fascicles, an examination was made of the foliage of 20 trees on each of the progeny plots. On each of these trees 10 fascicles were examined on each of 5 branches selected at random from the lower half of the crown. On each branch, approximately equal numbers of fascicles were examined on each of the internodes having green needles. A separate record was kept for each tree. For each plot the average percentage of fascicles containing three needles was determined, as shown in table 3.

Comparison of progeny trees with native trees as to the proportion of three-needle fascicles was made by the use of specimen branches obtained from the parent localities. As the foliage in any locality

may vary greatly among individual trees according to age of tree, exposure, and character and moisture content of the soil, and on a single tree according to position in the crown, collectors were requested to select outer branches below the middle of the crown on the south sides of vigorous trees, 20 to 40 years old, growing in open stands on good sites. Thus an effort was made to obtain foliage specimens from the same general position in the crown and from the same kinds of trees as on the progeny plots. From 3 to 10 specimens, representing that number of trees, were obtained from each locality. On each branch 50 fascicles were examined, a total of 150 to 500 for each locality. The results are presented in table 3.

TABLE 3.—*Percentage of fascicles containing three needles,¹ on progeny plots and in parent localities*

Climatic region and locality of seed origin	Fascicles containing 3 needles		Group character
	Progeny plot	Parent locality	
	Percent	Percent	
North Pacific: Siskiyou.....	93	100	
North plateau:			
Boise.....	95	99	
Payette.....	97	100	
Whitman.....	98	93	
Umatilla.....	98	100	
Colville.....	93	100	
Kaniksu.....	93	94	
Lolo.....	95	99	
Bitterroot:			
4,000 feet.....	97	95	Typically 3-needed.
5,000 feet.....	94	98	
7,200 feet.....	95	99	
South plateau:			
Coconino.....	94	97	
Santa Fe.....	92	96	
East of Continental Divide:			
Helena.....	69	88	
Custer.....	24	25	
Harney.....	24	7	
Roosevelt.....	11	22	Typically 2-needed.
San Isabel.....	51	43	
Central plateau: Ashley.....	60	76	Intermediate.

¹ All fascicles not containing 3 needles contained 2.

In all cases, the findings for trees in a parent locality and those for the progeny derived from that locality were similar, indicating that number of needles to the fascicle is an inherited characteristic persisting at least through the first 22 to 26 years of the progeny's life. The evidence from the areas sampled shows that in general three-needle fascicles are characteristic of ponderosa pine in the north and south plateau regions and two-needle fascicles are characteristic east of the Continental Divide. The Helena locality, close to the Divide on the east, is an exception. The Ashley locality, in the central plateau region, tends to be intermediate.

LENGTH AND PERSISTENCE OF NEEDLES

With regard to variation in needle length, Shaw (29, p. 4) says:

Among conifers, the leaf of *Pinus* attains extraordinary length with great variation, * * * the maximum for each species being usually much more than twice the minimum. Climate is the predominating influence; for the shortest leaves occur on alpine and boreal species, the longest leaves on species in or near the tropics.

The length of the leaf is complicated by the peculiarities of individual trees and by pathological influences; as a general rule, however, the length of leaves is less or greater according to unfavorable or favorable conditions of temperature, moisture, soil and exposure. Therefore the dimensions of the leaf may be misleading. It can be said, however, that certain species always produce short leaves, others leaves of medium length, and others very long leaves.

Data on needle length were collected not only on progeny trees but also on trees in the parent localities. The method of selection was to pluck at random 10 or more fascicles from 1 branch on each of 3 trees, taking a proportionate number from each of the internodes. Measurements were made of 30 or more needle clusters for each progeny plot, and 50 for each parent locality. Average length was calculated for the shortest 10 and the longest 10 to obtain "principal range," and for the total number of fascicles.

TABLE 4.—*Length of needles on progeny plots and in parent localities*

Climatic region and locality of seed origin	Progeny plot		Parent locality		Group character
	Principal range	Average	Principal range	Average	
North Pacific: Siskiyou	<i>Inches</i> 4.8-6.6	<i>Inches</i> 5.9	<i>Inches</i> 4.9-7.6	<i>Inches</i> 6.4	} Long.
North plateau:					
Boise	4.8-6.5	5.8	4.8-7.5	6.1	
Payette	4.4-6.8	5.6	4.4-6.6	5.7	
Whitman	4.2-6.9	5.9	4.8-6.7	5.9	
Umatilla	4.7-6.3	5.8	5.4-7.9	6.9	
Colville	4.7-5.8	5.4	5.1-6.6	5.7	
Kaniksu	4.7-6.2	5.6	5.0-7.1	5.0	
Lolo	5.8-6.8	6.3	5.1-7.4	6.1	
Blitterroot:					
4,000 feet	4.9-7.4	6.1	5.1-6.8	6.1	} Medium to long.
5,000 feet	4.3-6.7	5.6	3.5-6.3	4.8	
7,200 feet	4.0-5.9	5.0	3.7-6.0	5.0	
South plateau:					} Medium to long.
Coeurine	4.0-5.8	4.9	4.5-6.2	5.4	
Santa Fe	3.9-5.3	4.6	4.7-7.6	6.2	
East of Continental Divide:					} Short.
Helena	3.9-5.7	4.9	5.8-7.6	6.7	
Custer	3.0-4.2	3.7	4.4-6.8	5.7	
Harney	2.7-3.9	3.4	3.2-5.3	4.3	
Roosevelt	2.9-4.7	3.8	3.2-4.8	4.1	
San Isabel	3.2-5.0	4.2	4.2-6.0	5.2	
Central plateau: Ashley	3.2-5.2	4.3	3.2-5.2	4.2	

It appears from the data in table 4 that characteristics as to needle length were hereditary in the progenies studied, at least for the first 22 to 26 years of their lives; that is, that for 20 years and more the progeny have for the most part fallen into the same general classifications of long, medium, and short needles as did the trees in the parent localities. The data show that the needles of trees of the North Pacific and north plateau regions are long, that those of the south plateau are medium to long, and that those of the central plateau and of the localities east of the Continental Divide are short. The Helena trees are again an exception, their needles being medium to long, more like those of localities west of the Divide.

Data on persistence, or the number of years needles remain green on the tree, were obtained for the progeny plots by recording for each of 5 branches on 20 trees per plot the number of internodes having green needles. Corresponding data for trees of the parent localities

were obtained by examining all the specimen branches collected in each locality. These data are presented in table 5.

TABLE 5.—Number of years needles persist on progeny plots and in parent localities

Climatic region and locality of seed origin	Progeny plot		Parent locality	
	Principal range	Mode ¹	Principal range	Mode ¹
	Years	Years	Years	Years
North Pacific: Siskiyou	2-4	3	2-3	3
North plateau:				
Boise	3-4	3	4-5	4
Payette	3-4	3	4-5	4
Whitman	3-4	3	4-6	5
Umatilla	3-4	3	5-6	6
Colville	3-4	4	4-5	5
Kaniku	3-4	4	4-5	5
Lolo	3-4	3	4-5	5
Bitterroot:				
4,000 feet	3-4	4	4-5	4
5,000 feet	3-4	4	4-5	4
7,200 feet	3-4	3	4-5	4
South plateau:				
Coconino	3-4	3	4-7	6
Santa Fe	3-4	3	(²)	(²)
East of Continental Divide:				
Helena	3-4	3	4-5	5
Custer	3-4	4	4-6	5
Harney	3-4	4	3-4	4
Roosevelt	3-4	4	5-7	6
San Isabel	3-4	3	4-6	6
Central plateau: Ashley	3-4	3	6-9	8

¹ The value occurring in the greatest number of cases.

² Values estimated on basis of incomplete evidence.

³ Evidence available too incomplete to serve as basis for estimate.

Persistence is closely related to needle length. In general, where the growing season is short and rigorous, as at high altitudes, both shoots and needles are short. To compensate for this dual dimensional deficiency, needles must persist on more internodes than under more favorable conditions. Examples of high-altitude species having short needles persisting over a long period are *Pinus albicaulis* and *P. balfouriana*.

Needles persisted mainly 4 or 5 years in parent localities in the north plateau region, 6 years in the more severe Roosevelt and San Isabel localities, and 8 years in the rigorous Ashley locality; but needles on the progeny trees uniformly persisted only 3 or 4 years, regardless of origin. Thus it appears that, in ponderosa pine, needle persistence is not inherited, and that if the same area of leaf surface is maintained in the Priest River as, for example, in the Ashley environment this is done in some other way than by long retention of needles. The method of adjustment in this particular case is indicated by the fact that the length of the three internodes containing green needles on Ashley progeny trees averaged practically the same as the length of the eight internodes containing green needles on Ashley parent-locality trees.

It is interesting to note that in experimental plantations in Switzerland containing trees of different seed origin, Burger (5) and Nägeli (23) found needle persistence of Scotch pine and Norway spruce, respectively, to be uniform regardless of shorter or longer retention in different parent localities.

INTERNAL STRUCTURE OF NEEDLES

In order to ascertain what differences in needle structure exist among the progenies of this experiment, arrangement was made with J. H. Ramskill, professor of forestry in the University of Montana, to undertake cooperatively a microscopic study of the needles. Professor Ramskill was supplied with needles from each progeny plot and from each parent locality, collected according to methods of sampling already described. He made a great number of cross sections from these needles, studied 688 of them, and made photomicrographs of selected sections. Some of the structural characteristics found by Ramskill to be most consistent and most clearly heritable are here summarized.

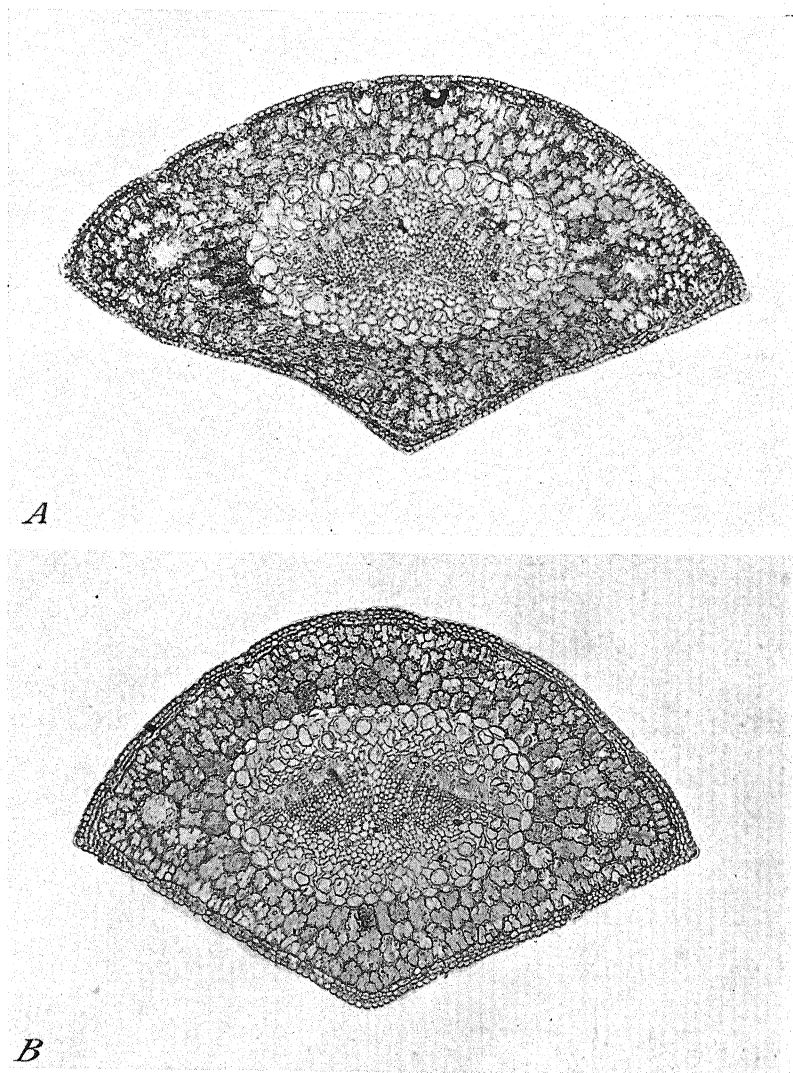
Leaf features in which plants are known to make protective adjustments in different habitats are thickness of hypoderm and character of stomatal chambers. The hypoderm in *ponderosa* pine is biform, having an outer row of cells (next to the epidermis) which is always thin-walled and an inner row or several inner rows of cells which may be thick-walled or thin-walled. In localities of severe climate, the inner rows may be many and composed solidly of thick-walled cells; in localities of moderate climate, they may be fewer in number and composed of thick-walled and thin-walled cells interspersed; in localities of mild climate there may be only one or two inner rows composed mainly of thin-walled cells, with a few interspersed thick-walled cells.

The observed extremes in these features are shown in plates 1, 2, and 3. It is readily seen in these photomicrographs that the hypodermal layer is composed of only a few rows of cells in needles from the Siskiyou locality, which has a mild climate, and of many rows in needles from the Ashley locality, which has a severe climate. Similarly the former are seen to have little depression of the stomata, whereas the latter have deeply depressed stomata. It is evident that the degree to which the openings of stomatal chambers are sunk below the general level of the leaf surface corresponds to the number of rows of cells in the hypoderm. Table 6 presents data on these two characteristics.

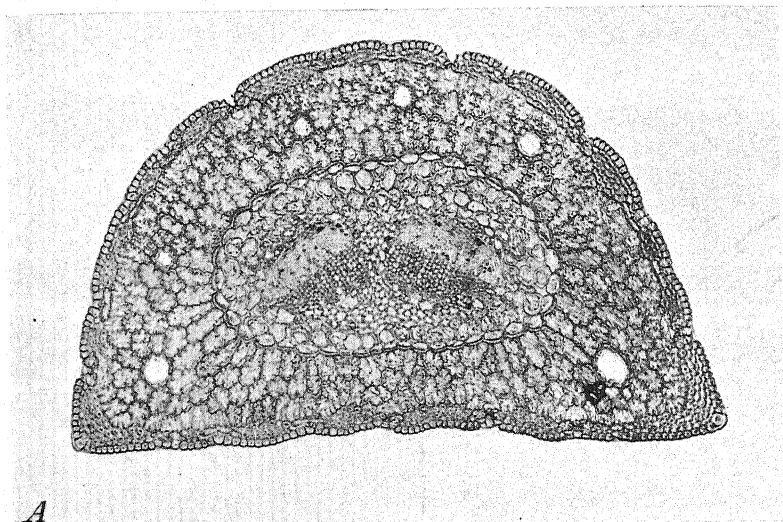
From the table and the plates it appears that, in general, hypoderm-cell and stomatal-depression characteristics have been inherited by the progenies in the new habitat and retained by them. A slight variation in degree of inheritance seems to be indicated in progenies derived from localities in the south plateau region and east of the Continental Divide, which have climates more rigorous than that of the experimental site on the Kaniksu Forest. Needles of these progenies tend to have somewhat fewer rows of hypoderm cells and slightly less stomatal depression than do needles in the parent localities.

When the localities are grouped by similarity of the hypodermal and stomatal characteristics described, as in table 6, they fall into three distinct main groups: (1) The North Pacific locality by itself, (2) the north plateau localities, and (3) all the localities of the central and south plateaus and the region east of the Continental Divide. Again the Helena locality is intermediate, resembling the north plateau localities more than the others.

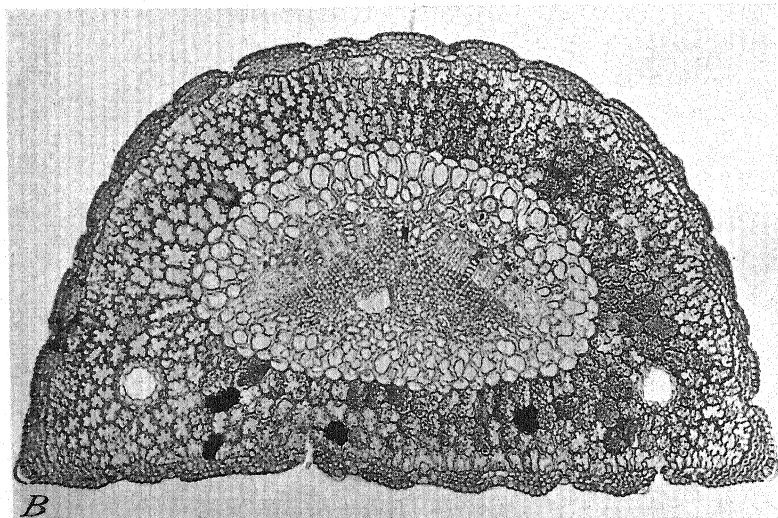
Table 7 presents data on two other features of needle structure—relative thickness of walls in inner rows of hypoderm cells and percentage of thick-walled cells in inner hypoderm rows. According to the available evidence, each of these characteristics may be regarded as inherited.



Cross sections of needles of Siskiyou trees, showing thin layer of hypoderm cells and little stomatal depression: *A*, Needle from parent locality; *B*, needle from progeny plot. Note the similarity between *A* and *B*, indicating inheritance of these characteristics in the new environment. These specimens are typical of the foliage of Siskiyou trees and closely resemble specimens typical of the foliage of north plateau trees. $\times 110$.



A



B

Cross sections of needles of Ashley trees, showing thick layer of hypoderm cells and deeply depressed stomata: *A*, Needle from parent locality; *B*, needle from progeny plot. Note the similarity between *A* and *B*, indicating inheritance of these characteristics in the new environment. These specimens are typical of the foliage of trees of the region east of the Continental Divide and the central and south plateau regions. $\times 110$.

TABLE 6.—*Number of inner rows of hypoderm cells containing thickened cell walls and depression of stomata in needles on progeny plots and in parent localities*

Climatic region and locality of seed origin	Inner rows of hypoderm cells containing thickened cell walls		Depression of stomata		Group characteristics	
	Progeny plot	Parent locality	Progeny plot	Parent locality	Rows containing thickened cell walls	Depression of stomata
North Pacific: Siskiyou.....	Number 1, 2	Number 1, 2	None.....	None.....	Few.....	None.
North plateau:						
Boise.....	1, 2, 3	1, 2, 3	Moderate...	None, moderate.	Few to moderate.	Slight to moderate.
Payette.....	1, 2, 3	1, 2, 3	do.....	do.....		
Whitman.....	1, 2	1, 2, 3	None, moderate.	do.....		
Umatilla.....	1, 2	1, 2, 3	Moderate...	do.....		
Colville.....	1, 2	1, 2, 3	None, moderate.	do.....		
Kaniksu.....	1, 2	1, 2	do.....	do.....		
Lolo.....	1, 2, 3	1, 2	do.....	Moderate...		
Bitterroot:						
4,000 feet.....	1, 2	1, 2, 3	do.....	do.....		
5,000 feet.....	1, 2, 3	1, 2	Moderate...	None, moderate.		
7,200 feet.....	2, 3	1, 2	do.....	do.....	Many.....	Moderate to deep.
East of Continental Divide:						
Helena.....	1, 2, 3	1, 2, 3	do.....	Moderate, deep.		
Custer.....	2, 3, 4	3, 4, 5	Deep.....	do.....		
Harney.....	2, 3, 4	2, 3, 4	do.....	Deep.....		
Roosevelt.....	2, 3, 4	3, 4, 5	do.....	do.....		
San Isabel.....	1, 2, 3, 4	2, 3, 4, 5	do.....	do.....		
South plateau:						
Coconino.....	2, 3	3, 4, 5	do.....	do.....		
Santa Fe.....	2, 3, 4	3, 4	do.....	Moderate...		
Central plateau: Ashley.....	2, 3, 4, 5	2, 3, 4, 5	do.....	Deep.....		

TABLE 7.—*Relative thickness of walls in inner rows of hypoderm cells and proportion of thick-walled cells in hypoderm of needles on progeny plots and in parent localities*

Climatic region and locality of seed origin	Relative thickness of cell walls ¹		Proportion ² of hypoderm cells having thick walls		Group characteristics	
	Progeny plot	Parent locality	Progeny plot	Parent locality	Cell-wall thickness	Cells having thick walls
North Pacific: Siskiyou.....	I, II	I	F, M	F, M	Mostly thin...	Few.
North plateau:						
Boise.....	II	I, II	M, A	M, A	Mostly thin to thick.	Moderate to many.
Payette.....	I, II, III	I, II	M, A	M		
Whitman.....	I, II, III	I, II	M	M, A		
Umatilla.....	I, II, III	I, II	M	A		
Colville.....	I, II	I, II	M	M		
Kaniksu.....	I, II	I, II	M	M, A		
Lolo.....	I, II	I, II	M	A		
Bitterroot:						
4,000 feet.....	I, II	II	M	M, A		
5,000 feet.....	II	II	M	M, A		
7,200 feet.....	II	II	M, A	M, A	Mostly thick.	Practically all.
East of Continental Divide:						
Helena.....	II, III	II	M, A	M, A		
Custer.....	II, III	II, III	A	M, A		
Harney.....	II, III	II, III	A	A		
Roosevelt.....	II, III	II, III	A	A		
San Isabel.....	II, III	II, III	A	A		
South plateau:						
Coconino.....	II, III	II, III	A	A		
Santa Fe.....	II, III	II, III	A	M, A		
Central plateau: Ashley.....	II, III	II, III	A	A		

¹ I=thin, or only slightly thicker than cell walls of epidermis; II=thick, or conspicuously thicker than cell walls of epidermis; III=very thick, or so thick as almost to eliminate lumen.

² As determined along perimeters of cross sections of needles. Includes all cells having thick or very thick walls. F=0-50 percent; M=51-99 percent; A=all, or 100 percent.

It will be noted that the localities fall into the same three groups on the basis of these characteristics as of those in table 6, and that the Helena locality is intermediate here also, with closer resemblance to the north plateau localities than to the others east of the Continental Divide.

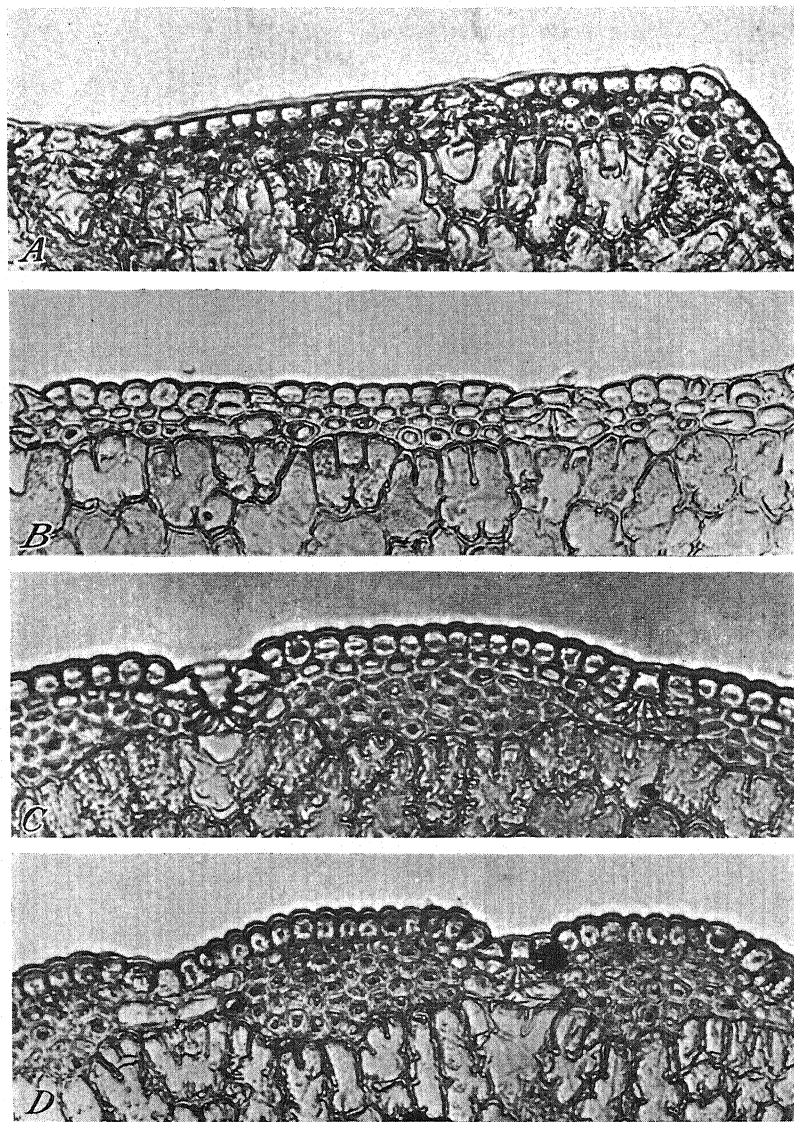
GENERAL APPEARANCE OF FOLIAGE

Even casual observers of the progeny plots have noticed many differences among the various progenies in general appearance of foliage. Corresponding differences are observed among the trees of the parent localities. The typical foliage appearance of the North Pacific and north plateau progenies is exemplified by the Umatilla progeny, illustrated in plate 4, *A*, and that of the progenies from east of the Continental Divide and from the central plateau by the Roosevelt progeny, illustrated in plate 4, *B*. Trees of the former group have relatively long, slender, flexible needles, typically occurring in fascicles of three, arranged on the branches in rather open plumes. Those of the latter have stiff, short, coarse needles, typically occurring in fascicles of two,⁶ arranged more compactly on the branches and in many cases curved toward the stem. The foliage appearance of the south plateau progenies, illustrated in plate 4, *C*, is intermediate.

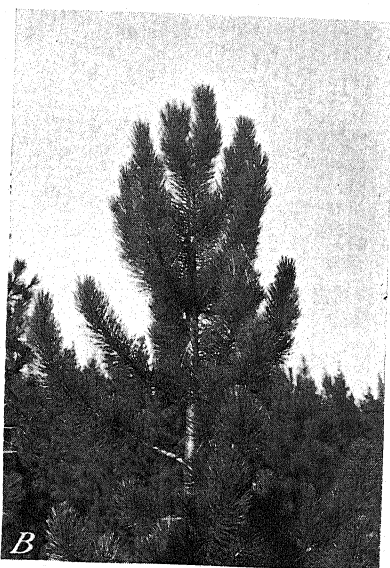
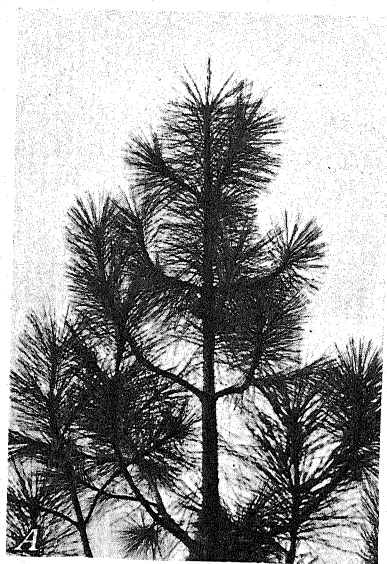
Thickness and stiffness of the needles are roughly proportional to quantity of stiffening tissue in the hypoderm, data on which are shown in tables 6 and 7. The foliage of the Coconino and Santa Fe stocks, in spite of heavy strengthening of the hypoderm, has an open appearance as compared with that of progenies or native trees of either the central plateau or the region east of the Continental Divide. This is accounted for by moderate length and relative slenderness of needles.

It is difficult to make distinctions as to color of foliage, because color is elusive and apparently varies to some degree with season, site, and health of the tree, and because the current year's foliage is often brighter in hue than the older foliage. In general, as seen from a distance, the foliage of the North Pacific and north plateau progenies is medium green to slightly yellow green and that of the progenies from east of the Continental Divide and from the central and south plateaus is gray green. On the basis of Ridgway's color charts (26), the progenies' foliage on the internode of the current season was classified in August 1936 as follows: Spinach green—Siskiyou, Kaniksu, Lolo, Bitterroot 4,000, Bitterroot 5,000, Bitterroot 7,200, Boise, Payette, Whitman, Umatilla, Colville, Coconino, Santa Fe, and Helena; light elm green—Roosevelt, Harney, Custer, and San Isabel; biscay green—Ashley. On the same basis their foliage on the internodes of earlier seasons was classified at that time as follows: Varney's green—Siskiyou, Kaniksu, Lolo, Bitterroot 4,000, Bitterroot 5,000, Bitterroot 7,200, Boise, Payette, Whitman, Umatilla, and Colville; deep dull yellow green—Coconino, Santa Fe, and Helena;

⁶ The Ashley progeny in this group, with only 39.8 percent of its fascicles containing two needles, may still be said to be strongly two-needled in character. In contrast, in all the progenies classified as three-needled, the fascicles containing two needles amount to less than 8 percent.



Sections of needles from Siskiyou (A) native tree and (B) progeny tree, characteristically containing few rows of hypoderm cells, and sections of needles from Ashley (C) native tree and (D) progeny tree, characteristically containing many rows of hypoderm cells. $\times 650$.



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Foliage characteristics of progenies: A, Open, plumelike arrangement of long, slender needles of Umatilla progeny, typical of the north plateau and north Pacific regions; B, compact, brushlike arrangement of short, thick needles of Roosevelt progeny, typical of the region east of the Continental Divide and the central plateau region; C, intermediate foliage characteristic of Santa Fe progeny, typical of the south plateau region.

pois green—Roosevelt, Harney, Custer, and San Isabel; light elm green—Ashley. A general correspondence in color was observed between trees of parent localities and progenies.

A purple bloom was found on tree branches collected in the Cocino and Santa Fe localities. On the progeny plots, in northern Idaho, a similar bloom appeared on twigs of Santa Fe, San Isabel, and Ashley trees but was not observed on Coconino trees.

GROUPING ACCORDING TO FOLIAGE CHARACTERISTICS

It is evident from the data presented in tables 3-7 that the localities of seed origin having similar foliage characteristics fall into a few groups, and that these groups are closely related to the climatic regions shown in figure 1. Table 8 summarizes these data.

It will be noted from table 8 that trees with preponderantly three-needle fascicles and long or moderately long, slender, flexible needles are found in the North Pacific, north plateau, and south plateau regions. Readily recognized differences in hypoderm structure, reflecting climatic differences, cause this group to subdivide into its three regional parts; hypoderm tissue is little or not at all thickened in the North Pacific trees, moderately thickened in the north plateau trees, and heavily thickened in the south plateau trees.

Trees having a preponderance of two-needle fascicles and distinctly short, thick, stiff needles with heavily thickened hypoderm tissue are typical of the region east of the Continental Divide.

In the Ashley and Helena localities foliage is intermediate in character. Needles of Ashley trees, in the central plateau region, are short, thick, and stiff and have heavy hypoderm structure, like those of trees east of the Divide. The central plateau trees resemble the south plateau trees in having more three-needle than two-needle fascicles. All factors considered, however, they are more closely related to those east of the Divide. The foliage of Helena trees is intermediate in needle length and hypoderm structure between typical foliage east of the Divide and that on the north plateau, but the similarity to the latter is much closer. This together with strong three-needle fascicle occurrence aligns the Helena trees with those of the north plateau. The Helena locality is only 15 to 20 miles east of the Divide, near enough to be affected by west side conditions.

Although no progenies from the South Pacific region are represented in this experiment, foliage data are available from specimen branches obtained from four trees at Quincy, in north central California. Collection and study of the branch material followed the procedure described for studying the foliage characteristics of trees in parent localities. The foliage of the Quincy trees had 99 percent of three-needle fascicles, and long, slender, flexible needles. Average length of needles was 7.3 inches, and principal range was 6.6 to 8.1 inches, longer than any measured in the experiment. Inner rows of hypoderm cells containing thickened tissue were few to moderate in number, the cell walls were only slightly to moderately thick, and stomata were but slightly to moderately depressed. All these characteristics are typical of the trees of the North Pacific and north plateau regions.

TABLE 8.—Summary of foliage characteristics by regions, based on progeny trees and trees in parent localities

Region or locality of seed origin	Typical appearance of foliage	Fascicles		Needle length			Hypodermal thickening
		General type	Having 3 needles	General type	Principal range	Average	
North Pacific.....	Needles long, slender, flexible. Foliage plumelike.	Typically 3-needled.....	Percent 90-100	Long.....	Inches 4.8-7.6	Inches 6.2	Very little.
North plateau.....	Medium green to yellow green.	do.....	90-100	do.....	4.6-6.9	5.8	Moderate.
Helena.....	Needles moderately long and slender, slightly stiff.	do.....	70-90	Medium to long.....	3.9-7.6	5.8	Moderate to heavy.
South plateau.....	Gray green.	do.....	90-100	do.....	4.0-6.9	5.4	Heavy.
East of Divide.....	Needles short, thick, stiff. Foliage bristly. Gray green.	Typically 2-needled.....	10-50	Short.....	2.9-5.7	4.3	Do.
Central plateau.....	do.....	Intermediate.....	60-75	do.....	3.2-5.2	4.2	Do.

GROWTH CHARACTERISTICS

HEIGHT AND DIAMETER OF PROGENY

The period from the first planting on a given progeny plot to the most recent measurement of the trees, in 1935, varied from 19 to 24 years. In order to compare all the progenies at a uniform age, heights and diameters were computed as of the end of the twentieth season after outplanting. As the nursery stock was in some cases 2 years and in others 3 years old at the time of outplanting, the total ages from seed represented by the height and diameter values thus obtained are 22 and 23 years.

In drawing conclusions from the growth figures, allowance should be made for the influences of density and spacing. All the plots are reasonably comparable in these respects except the Siskiyou, Bitterroot 7,200 feet, and Santa Fe, on which relatively few trees remain and these are widely spaced. As the surviving trees are the best of the individuals planted, at least on the first two plots, their growth is probably somewhat too advanced to be representative.

Table 9 shows for each plot the average height, the standard deviation of heights, and the extremes of height. Despite the uniformity of the site on which the trees are growing, there are wide differences in height growth. Average heights range from 15.7 feet for the Lolo to 7.2 feet for the Ashley progeny. In general the progenies derived from localities near the site of the experiment have made the best height growth. Those derived from the highest altitudes have in general made the least growth. These results are in agreement with those of European investigations (5, 8, 9, 12, 35).

TABLE 9.—*Height of progeny trees after 20 years' growth on plots*¹

Region, locality, and altitude (feet) of seed origin	Average	Standard deviation	Standard error	Maximum	Minimum	Basis, trees
	<i>Feet</i>	<i>Feet</i>	<i>Feet</i>	<i>Feet</i>	<i>Feet</i>	<i>Number</i>
North Pacific: Siskiyou (2,000).....	11.9	±4.5	±0.95	19.5	4.0	22
North plateau:						
Boise (5,500).....	10.4	±4.2	±.50	27.5	3.0	71
Payette (5,000).....	10.5	±2.7	±.40	19.0	3.5	45
Whitman (5,000).....	9.4	±3.7	±.65	20.0	4.0	33
Umatilla (3,500).....	11.7	±3.4	±.56	19.0	3.5	37
Colville (2,700).....	12.4	±4.1	±.44	21.0	4.5	86
Kaniksú (2,600).....	12.6	±3.9	±.50	21.0	1.5	61
Lolo (3,000).....	15.7	±4.9	±.54	26.0	6.5	81
Bitterroot:						
4,000.....	14.5	±4.1	±.51	22.0	6.0	65
5,000.....	13.1	±3.7	±.40	23.0	2.5	86
7,200.....	11.4	±4.6	±.76	21.0	3.5	36
East of Continental Divide:						
Helena (4,500).....	12.8	±3.4	±.36	21.0	6.0	87
Custer (3,200).....	9.8	±3.0	±.35	15.5	4.0	70
Harney (5,000).....	11.1	±4.9	±.55	17.0	4.0	79
Roosevelt (8,000).....	8.6	±2.6	±.50	15.5	5.0	27
San Isabel (8,000).....	9.0	±2.8	±.47	15.0	4.5	36
South plateau:						
Coconino (7,100).....	9.0	±2.6	±.33	15.5	4.5	63
Santa Fe (8,000).....	7.5	±2.4	±.54	11.0	3.0	20
Central plateau: Ashley (7,500).....	7.2	±2.6	±.37	12.0	1.5	49

¹ As the progeny trees were 2 and 3 years old when planted on the plots, the total ages represented by these measurements are 22 and 23 years.

Average diameters at breast height (4.5 feet above ground) 20 years after outplanting were read from height-diameter curves made for all plots (table 10). They ranged from 3.0 inches for the Lolo progeny to 1.2 inches for the Santa Fe and Ashley progenies. Table 11 presents average heights and diameters of dominant trees.

TABLE 10.—*Diameter of progeny trees after 20 years' growth on plots*¹

Region, locality, and altitude (feet) of seed origin	Average	Standard deviation	Standard error	Basis, trees
	<i>Inches</i>	<i>Inches</i>	<i>Inches</i>	<i>Number</i>
North Pacific: Siskiyou (2,000)	2.8	±1.5	±0.32	21
North plateau:				
Boise (5,500)	2.1	±1.4	±.17	68
Payette (5,000)	2.0	±1.3	±.19	44
Whitman (5,000)	1.8	±1.3	±.23	30
Umatilla (3,500)	2.2	±1.5	±.25	36
Colville (2,700)	2.4	±1.4	±.16	85
Kaniksu (2,600)	2.6	±1.2	±.15	58
Lolo (3,000)	3.0	±1.3	±.15	81
Bitterroot:				
4,000	2.9	±1.1	±.22	65
5,000	2.5	±1.2	±.13	85
7,200	2.3	±1.1	±.19	35
East of Continental Divide:				
Helena (4,500)	2.0	±1.2	±.13	87
Custer (3,200)	1.4	±1.1	±.13	70
Harney (5,000)	2.0	±.7	±.08	78
Roosevelt (8,000)	1.5	±1.3	±.24	27
San Isabel (8,000)	1.7	±1.2	±.21	36
South plateau:				
Coconino (7,100)	1.8	±1.2	±.15	63
Santa Fe (8,000)	1.2	±1.0	±.02	16
Central plateau: Ashley (7,500)	1.2	±.9	±.01	43

¹ See footnote 1, table 9.TABLE 11.—*Average height and diameter of dominants among progeny trees after 20 years' growth on plots*¹

Region, locality, and altitude (feet) of seed origin	Height	Diameter	Basis, trees	Region, locality, and altitude (feet) of seed origin	Height	Diameter	Basis, trees
	<i>Feet</i>	<i>Inches</i>	<i>Number</i>		<i>Feet</i>	<i>Inches</i>	<i>Number</i>
North Pacific: Siskiyou (2,000)	17.3	4.5	7	East of Continental Divide:			
North plateau:				Helena (4,500)	16.6	3.1	28
Boise (5,500)	17.2	4.2	13	Custer (3,200)	13.6	2.4	9
Payette (5,000)	13.8	3.1	11	Harney (5,000)	13.8	2.9	23
Whitman (5,000)	15.0	3.8	6	Roosevelt (8,000)	12.3	2.8	6
Umatilla (3,500)	15.3	3.4	11	San Isabel (8,000)	13.2	3.1	7
Colville (2,700)	17.4	4.2	26	South plateau:			
Kaniksu (2,600)	16.5	3.9	20	Coconino (7,100)	12.8	3.2	13
Lolo (3,000)	21.3	4.6	24	Santa Fe (8,000)	9.7	2.2	6
Bitterroot:				Central plateau: Ashley (7,500)	10.9	2.5	8
4,000	18.3	4.0	23				
5,000	17.3	3.7	22				
7,200	17.2	3.9	10				

¹ See footnote 1, table 9.TABLE 12.—*Regional averages of height and diameter of progeny trees after 20 years' growth on plots*¹

Region or locality of seed origin	Average height ²	Range of plot heights	Average diameter ²	Basis, plots	Region or locality of seed origin	Average height ²	Range of plot heights	Average diameter ²	Basis, plots
	<i>Feet</i>	<i>Feet</i>	<i>Inches</i>	<i>Number</i>		<i>Feet</i>	<i>Feet</i>	<i>Inches</i>	<i>Number</i>
North Pacific	11.9		2.8	1	East of Continental Divide	10.0	8.6-11.1	1.7	4
North plateau	12.5	9.4-15.7	2.4	10	South plateau	8.6	7.5-9.0	1.7	2
Helena	12.8		2.0	1	Central plateau	7.2		1.2	1

¹ See footnote 1, table 9.² Regional values shown are weighted averages of locality averages.

In table 12 a comparison is made among the regions as to average heights and diameters of progeny. Statistical analysis by the method of variance shows no significant differences in average height



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Stand views of progeny plots in 1935: A, Straight, tall, well-formed stems of Lolo progeny; B, slightly crooked and rapid-tapering stems, open branches, and rounded slow-growing tops of Coconino progeny.



General view of Bitterroot 5,000 plot in 1932, showing tree form and pointed crowns typical of north plateau progenies.

270,759

between North Pacific progeny (1 plot), north plateau progenies (10 plots), and Helena progeny (1 plot). On the other hand, the difference in average height between progenies derived from localities east of the Continental Divide (4 plots) and north plateau progenies is statistically significant, and so are the differences between south and north plateau progenies and central and north plateau progenies. Thus it is shown that the progenies derived from the south and central plateaus and the region east of the Continental Divide are truly of slow growth in northern Idaho.

RELATION OF HEIGHT TO DIAMETER

Another growth characteristic of interest to the forester is the height-diameter relation, which reflects capacity to make volume and quality growth. In the absence of data for the determination of form factors, height as related to diameter at breast height was taken from the curve made for each plot. The data are presented in figure 4.

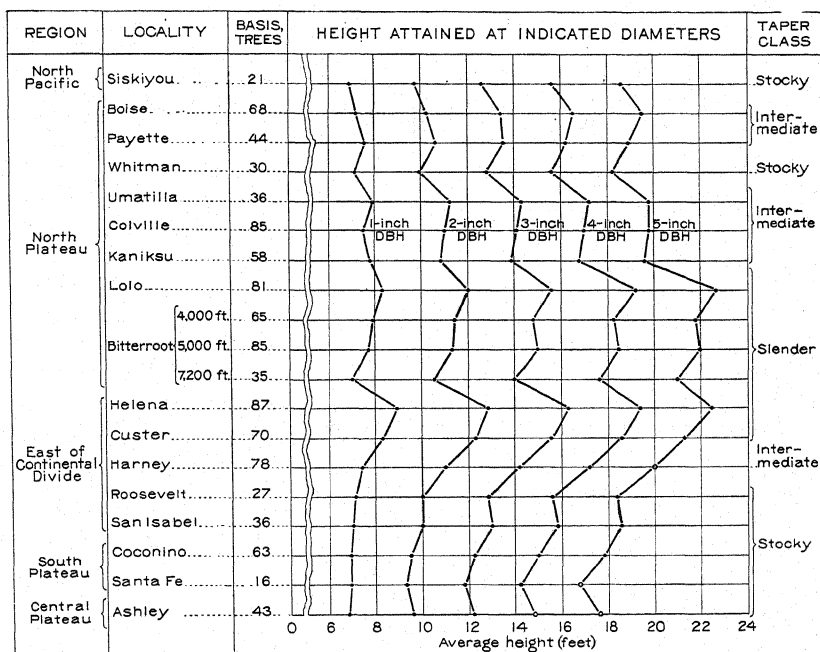


FIGURE 4.—Relation of average height of progenies to diameter in the various plots. (Dots indicate actual and circles interpolated data.)

Great differences in form are evident. For example, average height of trees 5 inches in diameter is 22.7 feet on the Lolo plot but only 16.8 feet on the Santa Fe plot. The range of differences was divided into three equal parts representing the three taper classes slender, stocky, and intermediate, of which slender is the most desirable and stocky the least desirable. Progenies in the slender class are the Lolo, Helena, Custer, and Bitterroot; those in the stocky class are the Santa Fe, Ashley, Coconino, Roosevelt, San Isabel, Whitman, and Siskiyou. Plate 5 presents examples of progenies having (A) well-formed stems and (B) poorly formed stems. Plate 6 shows the general form and

pointed crowns of a typical north plateau progeny. (Compare with pls. 4, *C*, and 5, *B*, illustrating the characteristic rounded crowns of typical south plateau progenies.) Attention is again called to the wide spacing of the surviving trees of the Siskiyou and Santa Fe progenies; it is not known what differences their stem forms would show if they had developed in closed stands.

It is believed that the data for ages 22 to 25 years represent the height-diameter ratios of most of the trees from youth to maturity. Because of comparatively early falling off of height growth in the Custer and Harney parent localities, however, it is reasonable to assume that the stem form of the two progenies will deteriorate later in life. On the other hand, the Whitman and Siskiyou progenies may be expected to continue height growth longer and improve in stem form, like the trees in their parent localities.

RELATIVE HEIGHT GROWTH BY YEARS

In studying introduced species and races, foresters both in Europe and in the United States have been inclined to judge the relative growth possibilities of different progenies from their behavior during

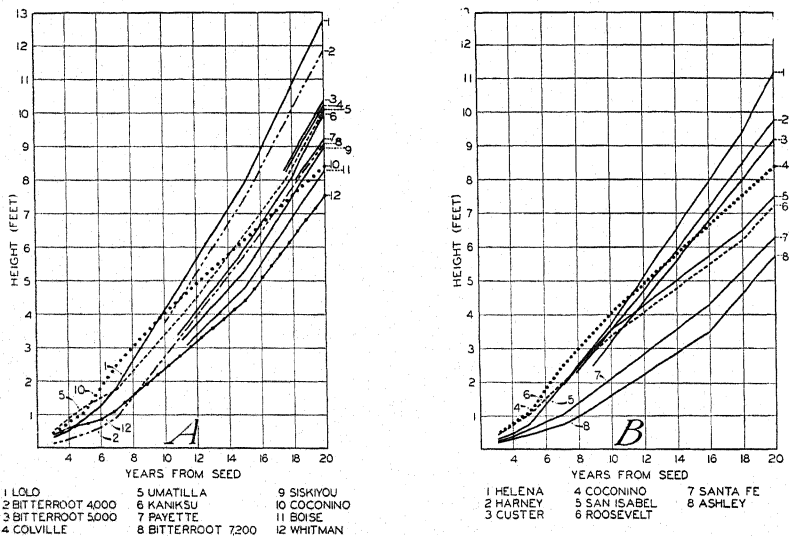


FIGURE 5.—Relative height growth, by year, of progenies derived (*A*) from North Pacific and north plateau sources, and Coconino; and (*B*) from south and central plateau and east of Continental Divide. Sources are indicated by number.

the first few years. This has not always been justified by the character of growth made in later years. Some introduced races that have made strikingly rapid height growth in the first few years have later fallen behind local races. The height measurements taken annually from 1912 to 1919 and those taken in 1927 and 1935 have made it possible to plot curves that show effectively the trends of early height growth in this experiment.

The height curves of the north plateau and North Pacific progenies and the Coconino progeny, given in figure 5, *A*, show that the Coconino progeny grew faster than any of the others during the first 10

years. Gradually, however, its growth rate has become less rapid, and local races have been overtaking it. The Lolo progeny, which in 1935 was growing more rapidly than any other, passed it at 10 years, the Bitterroot 4,000 at 11 years, the Umatilla at 14 years, and most of the others by 18 years.

Figure 5, *B*, shows that the Coconino progeny was foremost in height growth among the progenies derived from the central and south plateaus and from east of the Continental Divide, until the Helena, Harney, and Custer progenies overtook it between 11 and 15 years. The Roosevelt, San Isabel, and Umatilla progenies also excelled in height growth at the start but were soon overtaken by local progenies.

This comparison is evidence that initial growth rate of introduced races should not be accepted as presaging the later development of the trees. The trends shown here may, indeed, be taken as a warning not to apply too far into the future the present conclusions from results at 22 to 26 years of age.

GROWTH OF PROGENIES COMPARED WITH GROWTH IN PARENT LOCALITIES

If an experiment such as that described here shows, for example, that a given introduced race is much slower in growth than a local race, and therefore unsuitable for planting as a timber crop, this indication ordinarily meets the needs of the practicing forester. The findings are much more valuable, however, if they indicate further whether the growth rate of the introduced race is hereditary or is due to a difference in environment. As one of the purposes of this experiment is to determine what characteristics are heritable, an effort has been made to ascertain the growth rates of ponderosa pine in the parent localities.

Suitable growth data have been made available for the general localities of seed origin through a recent interregional yield study⁷ of even-aged ponderosa pine stands in the northern part of the range of the species, from the Black Hills to California. The data used in this yield study included average heights of dominant and codominant trees by decades for each of 13 site classes. From these data and from measurements yielding average site indices made in the general localities of seed origin, it was possible to plot the curves for this portion of the range shown in figure 6, *A*. As the basic data were intended solely for use in constructing yield tables, they were deficient in measurements of stands less than 25 years old. Therefore the lower portions of the curves had to be constructed by extension, and the heights indicated are close approximations only. These curves are sufficiently accurate, however, to serve in comparing rates of growth in the different localities. The curve shown in figure 6, *A*, for Arizona and New Mexico (south plateau) was plotted from growth measurements made by H. M. Curran, in his report already cited. Data on rates of growth are not available for the specific localities of seed origin in Utah, Colorado, or western Oregon.

To construct figure 6, *B*, average heights of dominant and codominant progeny trees at 20 years were plotted and a straight line was drawn from each plotted height to the origin point.

⁷ This study was supervised by W. H. Meyer, then of the Pacific Northwest Forest and Range Experiment Station, and the site-index data here used were supplied by him. The contribution to this study from the Northern Rocky Mountain Region consisted of data on 101 yield plots in northern Idaho, 99 in western Montana, and 35 in eastern Montana.

In studying figure 6 it will be seen that the curves representing the western Montana, northern Idaho, and southern Idaho stocks occupy the upper part of the growth range both of native trees and of progeny trees, and that the curves for eastern Montana, Black Hills, and south plateau stocks occupy the lower part of each range. The curve for the Arizona and New Mexico trees is lower than all the others in *A*, and conspicuously so in *B*.

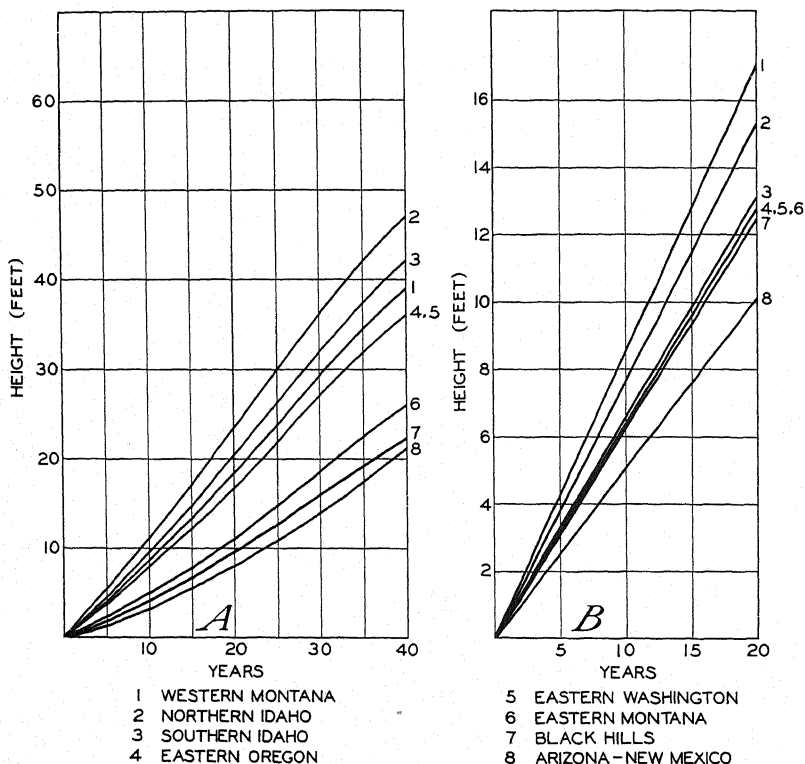


FIGURE 6.—Average heights of dominant and codominant trees (*A*) of some of the parent localities and (*B*) of some of the progeny plots. Seed sources are indicated by number.

It appears that progenies from localities of more severe climate than northern Idaho have inherited the slow growth rates typical of such localities. This agrees with findings of numerous European investigations (5, 6, 8, 12, 18, 35). As shown by the present study, the inheritance is strongly maintained through more than 20 years of the first generation. More extended experiments in Europe described by Dengler (9) and Münch (21) indicate that inherited growth characteristics are maintained also in trees of the second generation of introduced races.

In the single case (Siskiyou) of a progeny originating in a climate considerably milder than that of the experimental site in northern Idaho, the rapid growth rate of trees in the region of origin was not exhibited by the progeny. The site indices for the northern Sierra

territory, embracing the Siskiyou and Shasta seed-source localities (not included in fig. 6), are much higher than those for any other part of the range of ponderosa pine included in this study. The Siskiyou progeny trees surviving the severe winter freeze in 1924 that killed all the Shasta progeny have grown fast, but not so fast as trees in the parent locality. This indicates the inhibiting influence of climatic conditions more severe than those of the parent habitat. Büsgen and Münch (6), Dengler (9), and others have pointed out similar results of introducing races from a mild climate to a cold one. There is here, of course, no indication that progeny of the same Siskiyou parent trees would fail to inherit rapid growth rate if planted in localities of mild climate. Rate of growth is a quantitative characteristic that is modified by environment as well as by inheritance.

PHENOLOGICAL CHARACTERISTICS

Among the factors that have an important bearing on the adaptability of progeny to a given climate, in addition to needle structure, are the parent trees' characteristics as to time of beginning and ending seasonal growth. In this connection Büsgen and Münch (6) state:

Races of trees from regions with a short growing season and bad forest growth, when transplanted to mild situations, retain the short duration of their vegetation, fail to utilize the longer growing season, and so remain behind the indigenous local races. In general they come into leaf earlier, it is true, because less heat suffices for their vegetation, but they cease their growth early in the summer. When the transfer is in the opposite direction the trees seek to retain their inherited long vegetative period, grow on into the autumn and perish with frost.

That vigor of growth and susceptibility to disease as well as to frost are in large measure determined by inherited phenological habits has been shown by many investigators (5, 8, 9, 12, 18, 19, 20, 34).

Phenological observations have not been made on the progeny plots, but have been conducted for 8 years on native conifers near the experimental site. They show that the date when the buds on indigenous ponderosa pine begin to burst ranges from April 12 to May 25, averaging April 29. In the Coconino parent locality, Pearson (25) observed during a period of 3 years that buds of ponderosa pine begin to burst from May 15 to May 25. Thus the growing season of the Coconino locality begins about 3 weeks later than that of the Kaniksu locality. The fact that the Coconino progeny has a short period of vegetative activity presumably has been one cause of the comparatively small growth it has attained at 20 years (fig. 5, B). On the other hand presumably the long period of growth activity characteristic of trees in the North Pacific region, transmitted to the Siskiyou progeny, has been one of the causes of the frost damage suffered by the latter in northern Idaho.

GEOGRAPHIC RACES INDICATED

The data on foliage and growth characteristics obtained in this study, together with information on climate in different parts of the range of ponderosa pine, clearly point to the existence of several forms or races. The grouping of localities of seed origin by similar foliage characteristics and the discussion in that connection have already indicated general racial trends. It remains to present

evidence contributed by an examination of climate and growth characteristics. Fine distinctions are not attempted. It is realized that in addition to the characteristics studied, racial distinctions depend also upon differences in cones,⁸ bark, and wood, disease resistance, and other factors not dealt with in this study. The data most indicative of racial differences are shown in tables 8 and 12. In this connection, attention is directed also to figures 1 and 2 and to tables 1 and 2.

NORTH PACIFIC REGION

Trees of the North Pacific region differ from those of the remainder of the ponderosa pine range chiefly in that they are adapted to a mild, equable climate and are poorly equipped to withstand low temperatures. Their leaf structure is distinct from that of the trees of any other region studied, since very few of the hypoderm cells have thick walls. In this region extremely low temperatures are unknown, and the frostless season is longer and total precipitation much greater than in any of the other ponderosa pine regions studied. The fact that all the progeny trees derived from one of the two seed-source localities in this region and most of those from the other died as a result of the precipitous drop in temperature on December 15, 1924, from 45° F. to -12° F. in 20 hours, may be traced to the absence of protective hypodermal thickening. Both rainfall and precipitation effectiveness are high in the North Pacific region, and growth is consequently better there than in any of the other regions. The evidence seems to indicate that the ponderosa pine of the North Pacific region is a race by itself.

NORTH PLATEAU REGION

The foliage of the north plateau trees resembles that of the North Pacific trees in length, slenderness, and flexibility of needles and in occurrence of three-needle fascicles, but it has a distinct moderate hypodermal thickening, which presumably has a part in enabling the trees to resist severe low temperatures better than those of the North Pacific region. The progenies derived from this region suffered little loss from the freeze of December 15, 1924. Growing-season precipitation and temperature are favorable and growth in the parent localities is better than in any of the other regions studied except the North Pacific. The growth of many of the progenies greatly excelled that of progenies from other regions. On the basis of structural adaptation to low temperatures, the north plateau trees constitute a race distinct from the North Pacific trees.

REGION EAST OF CONTINENTAL DIVIDE

As already brought out, the external foliage characteristics of progeny and native trees of the region east of the Continental Divide differ conspicuously from all others in this experiment except those of the Ashley locality in Utah. The outstanding characteristics of the foliage of ponderosa pine in this region are the prevailing occurrence

⁸ Until 1936, cones were borne by the progeny trees only singly and sporadically. Counts in 1936 showed that 11 percent of the Helena trees bore 84 cones; 7 percent of the Roosevelt trees, 24 cones; 3 percent of the Umatilla trees, 9 cones; 2 percent of the Payette, 15 cones; 2 percent of the Kaniksú, 12 cones; and 2 percent of the Lolo, 3 cones.

of two-needle fascicles, the shortness, thickness, and stiffness of the needles and their compact arrangement on the branches, and the large number of rows of cells and heavy thickening of the cell walls in the hypoderm. Rate of growth is mostly slow. The progenies derived from this region have displayed a high degree of hardness (14). Altogether these distinctive characteristics make this a strongly demarcated race.

SOUTH PLATEAU REGION

In moderate length and slenderness of needles and in percentage of three-needle fascicles, the trees of the south plateau show relationship to those of the north plateau and North Pacific regions. The southern form, however, is distinctly different in its conspicuously heavy thickening of hypoderm cells, slightly stiff rather than flexible needles, and much slower rate of growth. The south plateau trees differ also from the trees typical of the region east of the Continental Divide, although internal leaf structure is the same in both forms. Existence of a distinct south plateau race is strongly evident.

CENTRAL PLATEAU REGION

The Ashley locality of seed origin, lying well within the central plateau region, has a lower annual precipitation than any of the other localities of the study, a pronounced deficiency of spring precipitation, and a generally low availability of moisture throughout the growing season. Its annual and summer averages of temperature are among the lowest. Thus precipitation and temperature together form a climate very rigorous for tree growth. This is reflected in the fact that the growth of the Ashley progeny, both in height and in diameter, was less than the average for any regional group of progenies. The needles are short, thick, and stiff and compactly arranged on the branches, as in the region east of the Continental Divide. Number of rows of hypoderm cells and thickness of the cell walls are the greatest found in this study. Foliage occurs mostly in fascicles of three, but many trees contain up to 40 percent of two-needle fascicles.

Thus the trees of this locality have a strong relationship to the trees east of the Continental Divide and have relatively little in common with those of the south plateau. In view of this, the Ashley trees are regarded as of the same race as those east of the Divide.

As indicated by Korstian (16) and by Baker and Korstian (2), the Ashley form of tree is found over much of Utah, Nevada, and the remaining territory included in the central plateau.

HELENA LOCALITY

In the Helena locality, 15 to 20 miles east of the Continental Divide, the foliage is strongly three-needled, with needles of medium length and moderate hypoderm thickening, thus resembling that of the north plateau more closely than that of localities farther east. The height growth of the Helena progeny, moreover, is as great as the average for north plateau trees and outstandingly better than that of other progenies derived from east of the Divide. Altogether, the evidence indicates so close a relationship that the Helena trees may be regarded as belonging to the north plateau race.

RACES SUMMARIZED

Briefly, the races tentatively indicated in this study and their important earmarks are the following:

1. *North Pacific race*.—Typically three-needled, needles long, very little thickening of hypoderm, rapid growth, relatively low frost resistance.

2. *North plateau race*.—Typically three-needled, needles long, distinct moderate thickening of hypoderm, good growth, good frost resistance.

3. *South plateau race*.—Typically three-needled, needles medium to long, very thick hypoderm structure, growth slow, good frost resistance.

4. *Race found east of Continental Divide and on the central plateau*.—Typically two-needled, needles short, very thick hypoderm structure, growth slow, highly frost-resistant.

Study of foliage from trees at Quincy, Calif., in the South Pacific region (no progeny available) shows fascicles very typically containing three needles. Needles are long and contain moderately thickened hypoderm. The data reveal a very close relationship to the North Pacific and north plateau trees, but are not adequate for ascertaining racial distinctions.

SUITABILITY OF RACES FOR PLANTING IN NORTHERN IDAHO

Decision as to the desirability of a tree race for introduction in a new locality involves consideration of both its adaptability to the local climate and its suitability for timber production. No matter how hardy and disease-resistant a race may be in the new locality, it will be useless for timber-crop production if it grows too slowly and develops a poor, stunted form.

Adaptability to climate unfortunately can be judged only by actual performance; and it has been learned by experience elsewhere that such performance, to be clearly indicative, must extend over at least one-third of the tree's life to maturity. The history of introduced races is replete with instances of break-downs of stands 25 to 40 years after planting. In the classic example described by Wibeck (34), many thousand acres of Scotch pine plantations in Sweden, derived from unsuitable seed sources, died out or developed poor form after having thrived well for 25 or more years. Similar happenings elsewhere in Europe have been reported by numerous writers (3, 7, 9, 11, 13). In South Africa, where the maritime pine (*Pinus pinaster*) of the Mediterranean countries proved to be an excellent species for introduction, Duff (10) shows that more than 285,000 pounds of maritime pine seed were imported between 1898 and 1914 with little or no regard for seed origin. Although many of the plantations have thrived and developed into stands of excellent growth rate and form, others, established with seed from unsuitable sources, at 40 years of age are of slow growth and stunted form or badly infected with disease. In the United States, although forest planting on an extensive scale is not so old, some plantations of introduced species that had an auspicious beginning have already broken down. These include several Scotch pine plantations in Pennsylvania⁹ that made exceedingly rapid, straight, and vigorous growth and gave high promise of being hardy

⁹ AUGHANBAUGH, J. E. SCOTCH PINE—AN ENIGMA. Pa. Dept. Forests and Waters Serv. Letter. 1935.

and adaptable until they were 20 to 25 years old, but then began to disintegrate badly through inability to withstand adverse conditions as to wind, snow, insects, and disease.

Thus it would be premature to make a statement on the ultimate adaptability to northern Idaho and adjacent territory of the progenies introduced in this experiment. Certain present indications of relative adaptability may be pointed out, however, with the warning that some future combination of climatic conditions may upset them.

The chief factors in climatic adaptability and suitability for timber growing are frost hardiness, disease resistance, rate of growth, and form. So far the progenies at the northern Idaho experimental site have not been seriously infected with disease and have developed only slight defects in stem form. They have, however, shown marked differences in growth rate and in relative resistance to untimely freezing temperatures.

It is well to keep in mind in this connection that individual parent trees may vary considerably in their ability to produce good or poor progeny in these respects. Although such differences between individual parents may account for some of the variation among trees of a given progeny plot in this experiment, it is believed that the plot averages are fairly safe to use in comparing the relative growth capacity and climatic adaptability of progenies derived from different localities.

The Siskiyou and Shasta stocks, which make rapid growth but are poorly equipped to resist frost, should not be considered for use in northern Idaho; and on the basis of extremely slow growth the Ashley, Roosevelt, San Isabel, Santa Fe, and Coconino stocks, with progeny heights averaging only 7.2 to 9.0 feet at 20 years, should also be excluded, despite the great hardiness of most of them. All the progenies other than those derived from the North Pacific region have withstood the severe freezes in northern Idaho during the period covered by this experiment, apparently because of their marked protective thickening of the hypoderm.¹⁰

According to the evidence obtained in this study, a progeny derived from a cold climate and grown in a milder climate exhibits slow growth and immunity to frost; a progeny derived from a mild climate and grown in a colder climate has low frost resistance and fails to exhibit the parental characteristic of rapid growth. Progenies introduced into the North Pacific region from any other part of the range of ponderosa pine would thus be expected to grow more slowly than native trees; and in the central and south plateaus and east of the Continental Divide, progenies introduced from the Pacific coast and the north plateau probably would suffer heavy losses by freezing and would grow more slowly than trees in their parent localities.

Such evidence as is available from similar experiments seems to confirm this reasoning. Near Carson, Wash., in the North Pacific region, in a 14-year-old experiment with ponderosa pine progeny,¹¹ offspring derived from the Bitterroot National Forest, Mont., grew very well but less rapidly than the local races, while progenies of Arizona, New Mexico, and Black Hills origin made the least height growth, as in the northern Idaho experiment. Near Manitou, Colo., in

¹⁰ Evidence of the effect of inherited hypodermal protection, in addition to that afforded by the freeze of December 1924, is the fact that trees in Savenac Nursery, in western Montana, grown from Custer seed, survived an October 1935 freeze with almost no sign of damage, whereas local stocks suffered some damage.

¹¹ Data supplied by the Pacific Northwest Forest and Range Experiment Station.

a 15-year-old experiment with ponderosa pine progenies,¹² the trees of local Roosevelt and San Isabel origin were hardiest and tallest, while those grown from seed derived from the Bitterroot (Mont.) and Tusayan (Ariz.) National Forests were shortest and most subject to losses. Only 5 and 12 percent, respectively, of the latter survived, and their height was only one-third that of the local progenies. Pearson (25) reported that first-year seedlings of ponderosa pine grown in a nursery near Flagstaff, Ariz., from seed collected on the Sierra National Forest, Calif., were completely killed by a November freeze, whereas those grown from seed collected in Arizona, New Mexico, Colorado, and the Black Hills were not injured. In an unpublished report he stated that ponderosa pine progenies grown near Flagstaff from seed collected in various localities in the northern part of the range of the species all died within 5 or 6 years.

Elimination of the Ashley, Roosevelt, San Isabel, Santa Fe, Cocconino, Siskiyou, and Shasta progenies as unsuitable for introduction in northern Idaho leaves 13 progenies, showing excellent to fair growth and having hardiness also in their favor, that must be given consideration as to suitability from a timber-growing standpoint. Ten of these are from localities in the north plateau region and three from localities east of the Continental Divide. Those making rapid height growth are, in order, the Lolo, Bitterroot 4,000, Bitterroot 5,000, Helena, Kaniksu, Colville, Umatilla, and Bitterroot 7,200. The Lolo and Bitterroot 4,000 progenies have a distinct lead over all others in height. The differences among the heights attained by the six other leading progenies are probably not significant, and for the present purpose all six may be regarded as equal in growth capacity. The slower growing of the north plateau progenies thus far are the Payette, Boise, and Whitman.

The Custer and Harney offspring are comparable in rate of growth with the slower growing of the north plateau progenies. As has already been pointed out, in the native localities of these two progenies the height growth of ponderosa pine falls off earlier than in the north plateau region; it is reasonably safe to assume, therefore, that in northern Idaho these progenies will fall far short of the ultimate height growth of trees of local origin. A strong point in their favor is high resistance to sudden drops in temperature below the freezing point (14). It is doubtful, however, whether this advantage can, even in the long run, compensate the faster growth of such local progenies as the Lolo, Bitterroot, Colville, and Kaniksu.

The Helena progeny shows not only a high degree of frost resistance but also a good rate of growth, comparable to those of the Kaniksu and Colville progenies. This indicates the Helena locality as a safe source of seed for planting in northern Idaho when local cone crops are inadequate.

Why the introduced Lolo and Bitterroot 4,000 progenies so definitely surpass the local Kaniksu stock in both growth and hardiness is difficult to explain on the basis of the data now available. Rain-fall and temperature records and soil-moisture tests shed but little light on the subject; and as has already been indicated, leaf structure is about the same for all three of these progenies. Perhaps the better part of wisdom would be to keep in mind the example of the rise and fall in height supremacy of the Coconino progeny, shown in figure 5,

¹² Data supplied by the Rocky Mountain Forest and Range Experiment Station.

and not attempt finely drawn explanations of the relative order of these north plateau progenies, on the basis of climatic or any other records.

It would appear that the most suitable general territory in which to collect seed for planting in northern Idaho (and western Montana) extends roughly from the Colville locality, in Washington, to a little beyond the Continental Divide, and from the latitude of the Salmon River approximately to the Canadian boundary.

SUMMARY AND CONCLUSIONS

Trees grown from seed of ponderosa pine collected in 20 localities in the western United States, widely separated geographically or in elevation, were planted on the Kaniksu National Forest, in northern Idaho, in the years 1911-17. Location and climate of the seed sources were known, but no data were recorded as to their soils or the individual characters of parent trees. Measurements of progeny trees were made in each of the years 1912-19, in 1927, and in 1935. At the time of the 1935 measurement the trees were 22 to 26 years old from seed.

A study of external and internal foliage characteristics of the progenies was made as a part of the 1935 examination of the plots. Differences in respect to number of needles to the fascicle, length of needles, general appearance of foliage, and thickening of hypoderm were found among progeny groups derived from five different regions within the range of ponderosa pine in the United States, which were delimited on the basis of precipitation type. These regions were designated North Pacific, north plateau, central plateau, south plateau, and east of the Continental Divide; the sixth region, the South Pacific, was not represented by any of the seed used.

Differences among the progenies in number of needles to the fascicle, length of needles, general appearance of foliage, and rate of growth corresponded to differences among the trees of the parent localities. The conclusion is drawn that these characteristics are strongly heritable in ponderosa pine and will appear in the offspring in any new environment where the trees will grow, at least for more than 20 years of the first generation.

Pronounced differences were exhibited by the different progenies in height and diameter growth. The slowest-growing progenies made only half as much growth as the fastest-growing. The best growth in height and diameter was made by trees derived from localities in the north plateau region resembling the locality of the planting site in climate. The poorest growth was made by trees derived from localities in Colorado, Utah, Arizona, and New Mexico that have more severe climates. Hereditary growth tendencies were less marked in the one progeny derived from a region having a climate considerably milder than that of the experimental area.

Characteristics as to persistence of needles were found not to be hereditary.

A study conducted cooperatively with the University of Montana revealed strong evidence that characteristics of internal structure of needles were inherited.

The present findings, revealing the existence of racial strains in ponderosa pine varying in rate of growth and hardness, indicate that a tree's growth rate and hardness should be investigated critically and the climatic characteristics of the locality in which it is

growing compared with those of the proposed planting site before the seed is used for reforestation. They indicate tentatively that the most suitable general territory in which to collect ponderosa pine seed for planting in northern Idaho is that extending from the Colville locality, in Washington, eastward a little beyond the Continental Divide and from the Salmon River to the Canadian boundary.

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THE ROLE OF INSECTS IN THE DISSEMINATION OF POTATO BLACKLEG AND SEED-PIECE DECAY¹

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INTRODUCTION

The important role of insects as vectors of plant diseases is one of the most striking developments in the field of phytopathology. This paper reports a study of the part taken by certain insects in the dissemination of blackleg (*Bacillus phytophthorus* Appel) and seed-piece decay in the potato (*Solanum tuberosum* L.), also some of the factors that influence the attack by insects and the consequent development of these diseases.

According to Leach (12, p. 150),³ "von Hegyi [in 1910], working in Polish Galicia and Prussian Silesia, found such a close correlation between blackleg and wire worm attack that he held biting insects to be a necessary factor for entry of the parasite." Jennison (7), who studied the problem in the United States, found the larvae of insects in and on the affected tissues but stated that there was no positive evidence that they were active agents in the dispersal of the disease.

Leach (11, 12) showed that the seed-corn maggot (*Hylemyia cili-crura* Rond.) has an active part in the spread and development of potato blackleg. Bonde (1) by means of controlled laboratory experiments confirmed Leach's conclusion. In later work Bonde, and also Reid et al. (17), showed that the penetration of the seed-corn maggot into potato seed pieces is often dependent on small, primary, bacterial lesions formed in the cut surfaces. Bonde (2) reported that the closely related seed-potato maggot, *H. (Phorbia) trichodactyla* Rond., may also transmit the disease.

Leach (13) found that two insects (*Scaptomyza graminum* and *Elachiptera costata*) are common agents of inoculation of celery heart rot. This disease is caused by *Erwinia carotovora* (Jones) S. A. B., the cause of blackleg and seed-piece decay in the potato. Johnson (9) and Bonde (3) demonstrated that the cabbage maggot (*Hylemyia brassicae* Bouche) may transmit a similar soft rot to members of the Cruciferae. The fact that several insects had been reported as being vectors of the soft-rot organisms led to further studies of the relation of insects to potato seed-piece decay and blackleg.

STUDIES WITH THE ANTHOMYIIDS

According to Leach (12) a symbiotic relationship exists between the pathogenic organism of blackleg and the seed-corn maggot, one of the anthomyiids. The eggs while being deposited in the soil may become contaminated by pathogenic bacteria, and the young maggots

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³ Italic numbers in parentheses refer to Literature Cited, p. 916.

may introduce this inoculum into the planted potato seed pieces. These pathogens and other microorganisms are present in the internal portion of the pupal and larval stages as well as on the surface of the eggs (15, 16).

HABITS AND LIFE HISTORY OF THE SEED-CORN MAGGOT (*HYLEMIA CILICRURA* ROND.)

The seed-corn maggot is widely distributed in both Europe and the United States (5, pp. 949-976). The literature on the economic losses caused by this insect is extensive. It shows that probably the larvae of this insect will attack practically any of the succulent and tender vegetable crop plants as well as grain, grasses, and weeds, provided conditions are favorable.

The life cycle of the seed-corn maggot under Maine conditions is not entirely known. In northeastern Maine the adults generally become prevalent in the spring at potato-planting time, especially after a few warm days. In 1932 flies from the overwintering puparia were emerging from the soil in great numbers on May 17, 18, and 19, following several days of warm weather. These flies, which are most abundant from the middle of May until the middle of June, appear to be attracted to the moist and freshly turned soil and are especially active following a warm rain. They soon commence to deposit eggs in the soil. Within a few days the eggs hatch, and the small larvae begin to feed on nearby vegetable matter. Under the conditions in Aroostook County the larvae are full grown within from 7 to 12 days, at which time they leave the vegetable substratum, enter the nearby soil, and pupate. On June 16, 1932, maggots that were full grown and about to pupate were found in decayed seed pieces. The adult insects from the overwintering puparia had practically disappeared by June 21, and the second brood was emerging. These also disappeared within 1 or 2 weeks.

The adult as well as the other stages may be noted to some extent during the entire summer. However, during July and early August the number of adults greatly diminishes; but usually they become quite numerous again the latter part of August and in September, and larvae have been found in decaying potato tubers affected with late blight. Potato tubers affected with late blight rot, followed by bacterial organisms, serve as a favorable host for *Hylemyia cilicrura* in late summer in northern Maine.

Puparia have been seen in the latter part of September and in early October. These late puparia apparently overwinter in the soil. The writer has overwintered puparia in soil in the field and reared them to adults the following spring.

The writer has not been able to demonstrate that the adult flies are attracted to decaying potato tubers and foliage. He has exposed healthy and decayed seed pieces to fly infestation in the field. Flies, although numerous, were not attracted more by the rotted tubers than by healthy ones.

In order to test the preference of the flies toward potato material, freshly cut seed pieces and uncut tubers were alternated in the field and exposed to flies under natural conditions. Notes were taken of the number of flies that visited the 2 lots of seed in 10-minute periods. An average of 62 anthomyiids visited the surfaces of the freshly cut

seed, and only 10 visited the uncut tubers. The flies apparently were attracted by the moisture and possibly by the odor of the freshly cut tubers. The flies are also attracted to fish and cottonseed meal when used as bait in insect traps exposed in the field.

During the egg-laying period the female flies are seen about potato seed pieces, clods, stones, or organic debris lying on the plowed fields, apparently in search of sheltered nooks or crevices for depositing their eggs. The female pushes the surface soil away with her hind legs, inserts her extensile ovipositor into the moist soil, and deposits the small, white, crescent-shaped eggs just beneath the surface sometimes on or near the planted potato seed pieces. Figure 1 shows eggs that

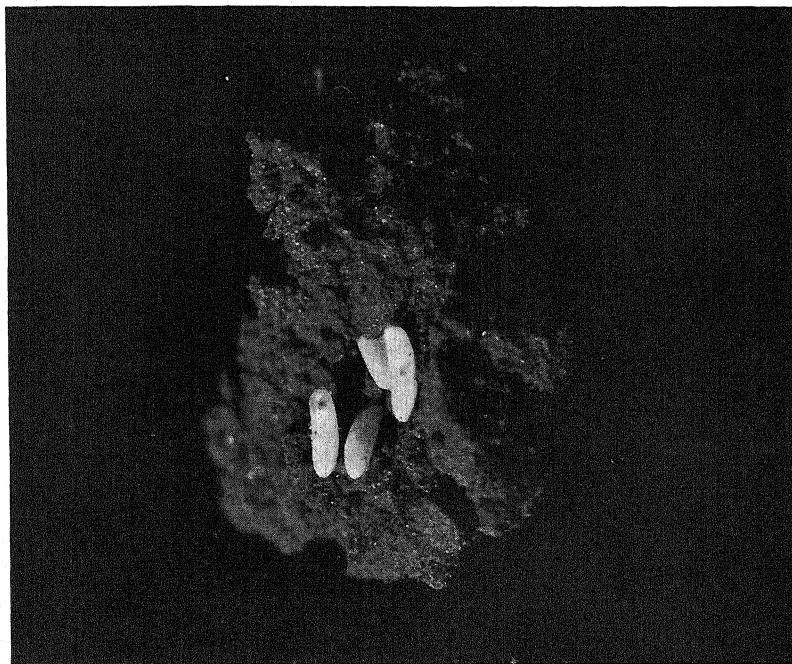


FIGURE 1.—Eggs of the seed-corn maggot that were deposited in the soil in a field. Approximately $\times 15$.

were deposited in the soil in a field. From several to a dozen or more may be deposited at a place.

SEED-POTATO MAGGOT (*HYLEMYIA TRICHODACTYLA* ROND.)

Johannsen (8) found the seed-potato maggot (*Hylemyia trichodactyla* Rond.) infecting a lot of seed potatoes from Aroostook County. He states that as this insect has a wide distribution and is fairly common, it is not unlikely that it is of economic importance. The writer has reared this insect from rotted potato seed pieces obtained from the field and has caught the adults by sweeping with a net and by using baited traps.

Although the seed-potato maggot possibly has a wide range of hosts, the writer has obtained it only from decaying potato material

and has caught it only in potato fields. The seed-corn maggot, on the other hand, has been secured in Aroostook County not only from potatoes, but also from seedlings of corn, beans, peas, and cucumbers. The data at hand would suggest that *Hylemyia trachodactyla* has a more specific preference for potato tissue than has *H. ciliatella*.

Examination of the flies caught with an insect net, or with baited traps, suggests that the seed-potato maggot is more prevalent in Aroostook County than the seed-corn maggot, especially in the vicinity of potato fields. Studies also indicate that the life history of these two insects is essentially the same.

ASSOCIATION OF PATHOGENIC BACTERIA WITH DIFFERENT STAGES OF THE SEED-CORN MAGGOT

Leach (12) found that pathogenic bacteria could be isolated from the different stages in the life cycle of the seed-corn maggot. He (16, p. 160-161) also demonstrated that the bacteria "survive in the lumen of the midintestine, in the cast-out linings of the fore- and hind-intestine, and in the space between the prepupal cuticle and the true pupa."

Although the writer has made no attempts to determine the exact place where the bacteria are harbored within the insect, he has made isolations from the different stages, which confirm Leach's results.

Leach showed that the surface of the egg may become contaminated in the process of oviposition. In 1929 the present writer secured only 1 pathogenic organism from the surface of eggs in 16 attempts, while in 1930 he secured 3 in 10 attempts (table 1). The adults were induced to deposit their eggs in pans of sterilized soil in the laboratory, and the eggs were removed with a moistened sterile needle to tubes of beef broth. The cultures were incubated until bacterial development was apparent and then tested for pathogenicity on potato slices.

TABLE 1.—Isolation of pathogenic bacteria from the different stages in the life cycle of the seed-corn maggot, Presque Isle, Maine¹

Year	Lot No.	Stage of insect	Source of insect	Total	Pathogenic		Types of decay
				Number	Number	Percent	
1927	1	Pupa.....	Laboratory ²	5	1	20	White.
	1	Egg.....	Deposited in sterile soil.....	16	1	6	White, rapid.
	2	Adult.....	Captured in field.....	28	2	7	Dark.
1929	3	Pupa.....	Decayed pea seed.....	32	7	22	White, rapid.
	4	do.....	Laboratory ²	38	16	42	Do.
	1	Egg.....	Deposited in sterile soil.....	10	3	30	Do.
1930	2	Adult.....	Captured in field.....	14	1	7	Dark.
	3	do.....	do.....	20	2	10	Do.
	4	Pupa.....	Potato seed pieces.....	30	2	7	White, rapid.
	5	do.....	Decaying beans.....	11	2	18	Slow, dark.
	6	do.....	Laboratory ²	6	1	17	White, rapid.
1931	1	do.....	Potato seed pieces.....	38	0	0	None.
	2	do.....	Reared in laboratory ³	14	1	7	White, rapid.
			Overwintered in field.....	14	1	7	Slow, dark.
1932	1	Adult.....	Captured in field.....	22	2	9	Dark, slow.
	2	Larva.....	Potato seed pieces.....	4	0	0	None.
	3	Pupa.....	Late blight tuber decay.....	32	2	6	White, rapid.

¹ The different cultures isolated from the respective insects were tested for pathogenicity on potato slices in moist chambers.

² From insects used in laboratory infection studies recorded in table 4.

³ Pupae reared in the laboratory and later overwintered in the soil in the field.

Bonde (2) in previous studies isolated soft rot bacteria from within the puparia of the seed-corn maggot. As shown in table 1, pathogenic bacteria were secured from the pupal stage in each of the five seasons in which the studies were conducted. Puparia containing pathogenic bacteria were obtained from the vicinity of potato tubers decayed by the late blight fungus and by secondary organisms.

The insects used in the infection studies (table 4) were secured from the field and produced blackleg and bacterial decay under controlled laboratory conditions. The pathogenic organisms isolated from these puparia were tested further on potato plants and found capable of causing blackleg.

As shown in table 1, the adult *Hylemyia cilicrura* may harbor pathogenic bacterial organisms. Leach concluded that the digestive tract of the adult insect contains the soft-rot bacteria and that the eggs in the process of oviposition are contaminated. The fact that the writer secured the organism from the surface of eggs deposited in sterile soil confirms this conclusion.

The writer has not isolated pathogenic organisms from the larval stage of *Hylemyia cilicrura*. His tests were, however, very limited and are not conclusive.

Isolations were made from the different stages of the seed-corn maggot reared in the process of experiments conducted in Charleston, S. C., during the potato-planting season of 1930. These isolations (table 2) were generally in accord with the results secured from the Maine studies.

One pathogenic bacterium isolated from a puparium reared in the laboratory is of particular interest. Maggots penetrated potato seed pieces planted in sterilized soil and produced an active decay. The pathogenic organism associated with this decay was reisolated from within a puparium reared from this material. This bacterium was found to be very actively pathogenic and capable of producing seed-piece decay and blackleg in the potato.

TABLE 2.—*Isolations of pathogenic bacteria from the different stages in the life cycle of the seed-corn maggot, Charleston, S. C., 1930*

Stage of insect	Source	Pathogenicity of cultures		
		Total	Pathogenic	
		Number	Number	Percent
Egg.....	Deposited in sterile sand.....	17	3	17.6
Adult.....	Reared from laboratory infection experiments.....	11	2	18.1
	(Decaying seed potatoes from field.....)	21	4	19.0
Pupa.....	Decaying spinach (<i>Spinacia oleracea</i> L.).....	2	0	
	Decaying chickweed (<i>Stellaria media</i>).....	2	1	50.0
	Reared from laboratory infection studies in sterile sand.....	17	1	5.8

The data in tables 1 and 2 have been summarized in table 3. Approximately 7 percent of the eggs, 15 percent of the puparia, and 10 percent of the adults harbored bacteria capable of rotting potatoes. The percentage of the insects containing pathogenic bacteria probably depends to a large extent on the type of rotting material in which the larvae fed. In some types of decay in which the bacteria are mostly saprophytic none of the insects may be carriers of pathogenic organisms. On the other hand, if the larvae have been feeding on material

decayed by the soft-rot bacteria, a high percentage of the insects may contain pathogenic bacteria.

All of the insects studied yielded, in addition to the pathogenic bacteria, an abundance of saprophytic bacteria of several kinds. None was free from microorganisms.

TABLE 3.—Summary of pathogenic organisms isolated from the different stages in the life cycle of the seed-corn maggot

Stage of insect	Total eggs or insects	Eggs or insects yielding pathogenic organisms	
	Number	Number	Percent
Egg.....	43	7	16.3
Larva.....	4	0	0
Pupa.....	257	38	14.8
Adult.....	95	9	9.5

BACTERIA AS A FACTOR IN THE NUTRITION OF THE SEED-CORN MAGGOT

The writer has not made a detailed study of the nutritional requirements of the seed-corn maggot. This problem has, however, a direct bearing on the feeding habits of the insect as related to injury to potato seed pieces. Leach (12) showed that sterile maggots were not able to develop on sterile potato tubers but grew normally when bacteria were added. Huff (6), who made a more detailed study of the nutritional requirements of the seed-corn maggot, found that the larvae would not grow to maturity on sterile beef-extract agar or potato plugs. When the agar or the potato plugs were contaminated with bacteria the maggots grew normally. He was unable to grow the larvae on bacteria-free filtrate from unheated potatoes or on sterile potato to which had been added a suspension of bacteria killed by heat; on the other hand, the larvae grew normally on potato plugs, beans, and peas that had been partly decomposed by bacteria and then sterilized by heat. Huff concluded that the bacteria per se are not essential for the development and pupation of this insect but that they convert plant tissue into available food for the larvae. Huff was able to rear the maggots at a normal rate of development on sterile bean and pea seedlings. Leach (15, p. 402) found that larvae would develop faster and a higher percentage would reach maturity if the seedlings were contaminated with bacteria.

The exact role of the bacteria found associated with the feeding larvae is not known. The writer has attempted to rear sterilized larvae on sterile potato-dextrose agar, beef-extract agar, and sterile cooked peas, beans, and cabbage. None of the attempts with these media were successful. The larvae also failed to develop normally on pure cultures of *Erwinia carotovora*, *Phytophthora campestis*, and *P. lachrymans* grown on the above-mentioned substrata. The larvae grew to some extent on the inoculated media, but in no case was the development rapid; and only a few puparia were formed when pure cultures of bacteria were employed.

The addition of a slight amount of unsterilized soil from the field greatly hastened the development of the larvae. When unsterilized soil was added to the different media in flasks in which the pure bacteria were growing, bacterial contamination occurred and thereafter

practically 100 percent of the eggs and larvae developed to the adult stage. The amount of soil added was too small to seem to have any direct nutritive value. These results indicate that certain other soil organisms found in association with the soft-rot condition are essential for the normal growth and development of the seed-corn maggot.

INOCULATION STUDIES WITH THE SEED-CORN MAGGOT AND SEED-POTATO MAGGOT

No evidence is available to show that the flies of the seed-corn maggot infect potato seed pieces by direct contact. The flies are often seen resting on and about seed pieces that have been left exposed in the furrow, but there is no proof that bacterial rots have resulted from such association. Observations both in Aroostook County, Maine, and at Charleston, S. C., failed to show that the adult of *Hylemyia cilicrura* Rond. frequents the potato bin. The writer has never seen a specimen of the adult in a potato bin or storage house, although he has made a special effort to observe adult flies about storage houses. Experiments were made to test the ability of *Hylemyia cilicrura* and *Hylemyia trichodactyla* to inoculate potato seed pieces by contact in the field.

STUDIES IN MAINE

Adult anthomyiid insects were captured in the field by means of wire-screen traps baited with moistened fish meal (fig. 2).^{4 5} The insects were then transferred to glass jars containing freshly-cut potato seed pieces partly covered with sterilized soil; for most of these studies from 5 to 10 adult insects were introduced into each jar with the seed piece, except in 1930 when a single fly was used in each jar. The seed pieces used in the studies of 1929 and 1930 were inoculated with pure cultures of *Alternaria solani* before being sub-

TABLE 4.—*Inoculation of potato seed pieces with adult insects in the laboratory*

Year	Experiment ¹ No.	Insect used	Development insects	Total seed pieces	Results of inoculation		
					Black- leg	Decay	Healthy
1927	1-----	<i>Hylemyia cilicrura</i>	Larvae numerous.....	Number	Number	Number	Number
	2-----	do.....	do.....	5	4	1	0
	3-----	do.....	do.....	5	2	3	0
	4-8-----	do.....	do.....	5	4	1	0
	9-----	<i>Drosophila</i> sp.....	do.....	25	0	0	25
	10-----	do.....	do.....	5	0	0	5
	Controls.....	do.....	do.....	5	0	0	5
1928	1-18-----	<i>Hylemyia cilicrura</i>	None.....	10	0	0	10
	1-11-----	do.....	Larvae numerous.....	90	0	0	90
1929 ²	12-36-----	do.....	None.....	55	22	22	11
	Controls.....	None.....	do.....	125	0	0	12
	1-5-----	<i>Hylemyia cilicrura</i> ³	Larvae present.....	5	0	0	55
	6-10-----	do.....	do.....	25	10	0	15
	11-----	do.....	None.....	25	0	0	25
1930 ²	12-13-----	<i>Hylemyia trichodactyla</i> ³	Larvae present.....	5	1	1	∞
	Controls.....	do.....	do.....	10	0	0	10
	Controls.....	None.....	do.....	10	0	0	10

¹ 5 seed pieces were included in each test.

² Seed pieces were inoculated with cultures of *Alternaria solani* and then transferred to the jars of sterilized soil with the insects.

³ 1 female fly was used for each jar.

⁴ The identification of the insects was kindly verified by Dr. O. A. Johannsen of Cornell University, Dr. H. C. Hockett of the Long Island Vegetable Research Farm, Riverhead, N. Y., also rendered aid.

⁵ The technique for obtaining flies of the seed-corn maggot was adopted from that used by W. J. Reid. The writer is indebted to him for help and courtesies shown in Charleston, S. C.

jected to the attack of the insects. This fungus was used because it causes shallow and superficial lesions on potato slices, and because such lesions attract the maggots. After shallow lesions had been formed by the fungus, the seed pieces were transferred to jars of sterilized soil, before the flies were introduced. The soil was kept

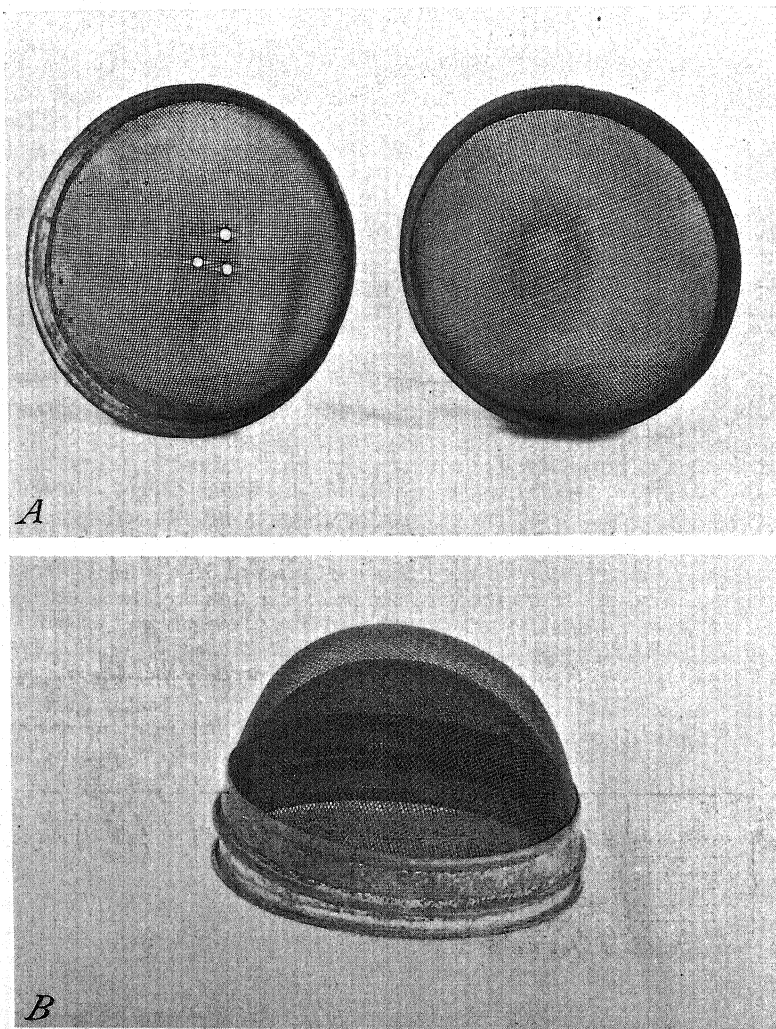


FIGURE 2.—Cage used for catching the adult of the seed-corn maggot in the field: A, Trap unassembled; B, trap assembled. These traps were baited with fish meal and set in the fields frequented by the insects.

moist by artificial watering. The temperature varied in the laboratory from 10° to 18° C. The results are summarized in table 4.

In 1927 daily observations were made for a period of about 8 days with no obvious decay being apparent. After approximately 21 days decay was present in 3 of the 8 jars. The rotted seed pieces,

contained numerous maggots of *Hylemyia cilicrura* which were actively feeding and burrowing into the potato tissue, and 5 of the 15 seed pieces disintegrated before plants were developed. The other

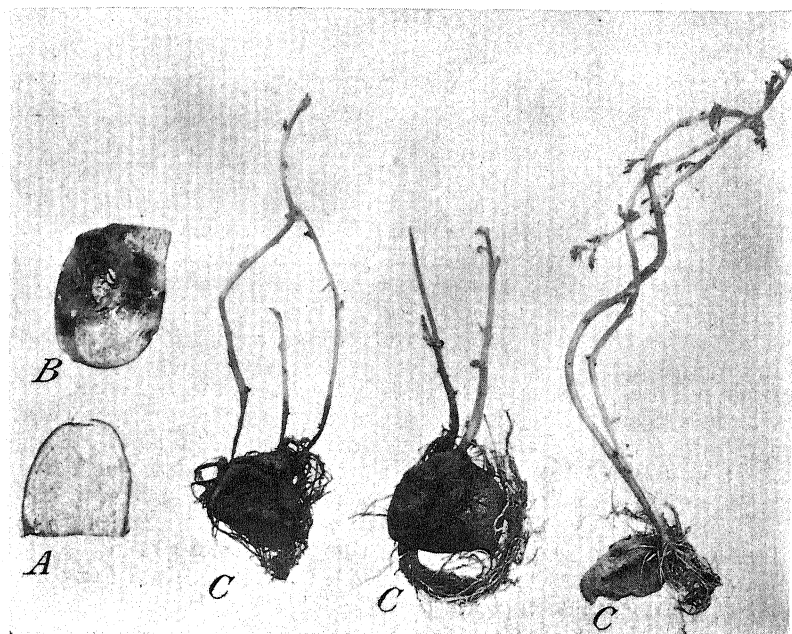


FIGURE 3.—Plants with the blackleg disease produced by inoculation with the seed-corn maggot in jars of sterilized soil: A, Healthy control; B, decayed seed piece showing maggots; C, diseased plants from which the blackleg organism was isolated.

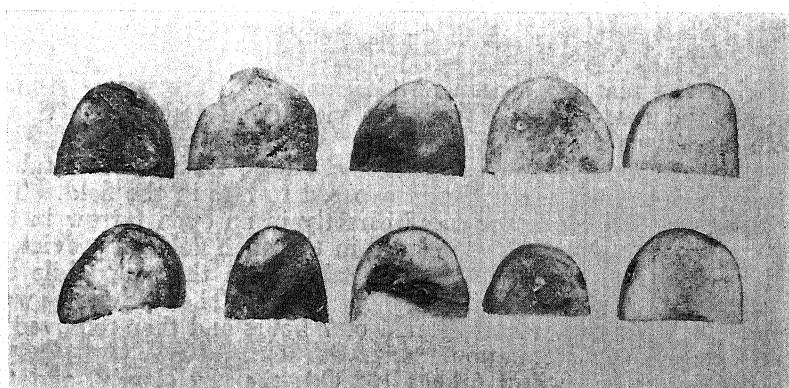


FIGURE 4.—Bacterial decay induced into healthy seed pieces by the seed-corn maggot. The healthy seed pieces were planted in jars of sterilized soil and the insects that had been caught in the field introduced.

10 seed pieces, less severely attacked by the soft-rot organism, produced plants with typical blackleg (fig. 3). Figure 4 depicts a bacterial decay induced in healthy seed pieces in a similar experiment,

also using the seed-corn maggot. Isolations were made from these affected plants, and a white-rot organism capable of producing the blackleg disease was obtained. The control seed pieces from the same tubers remained sound until discarded late in the season.

In the five jars (Nos. 4-8) in which decay failed to develop, the adult insects perished. Larvae were produced but were unable to penetrate the tissues of the healthy seed pieces and therefore died in an early stage of development.

In 1928 all of the 18 insect inoculations gave negative results.

The experiment was repeated in 1929. The results are given in table 4.

In these infection studies inoculated seed pieces oftentimes failed to decay and produce diseased plants. There may be several reasons for these failures: Some of the flies apparently were infertile when captured in the field; moreover, maggots seldom attack seed pieces unless bacterial lesions or a superficial decay are present. The reasons for this will be explained later. Also, as shown in a preceding section of this paper, many maggots are not contaminated with pathogenic bacteria and thus are not effective agents of inoculation.

In 1926 insects reared from decaying potato seed pieces secured from different fields were all identified as being *Hylemyia trichodactyla*. It was concluded that possibly this species also was a factor in the transmission of seed-piece decay and blackleg in Maine.

Examination of the insects caught by sweeping with a net about gardens and fields revealed that often these two species of anthomyiids was obtained. In 1930 it was decided to use individual female flies for the insect-inoculation studies.

The data obtained from a series of inoculation experiments with individual flies in 1930 are recorded in table 4. The data, although not very extensive, indicate that *Hylemyia trichodactyla* is also capable of introducing the soft-rot bacteria into healthy potato seed pieces and thus initiating blackleg.

STUDIES IN CHARLESTON, S. C.

During the planting season of 1929 and 1930 the writer studied blackleg and seed-piece decay in the vicinity of Charleston, S. C., where conditions are quite different.

Freshly cut seed pieces from surface-sterilized Irish Cobbler tubers were divided into several lots and exposed to flies in the field. The seed pieces of one lot were placed directly in an open furrow in the soil, those of another lot were placed in the open field on previously disinfected papers to avoid contamination from the soil, while the controls were maintained in covered containers. The seed pieces were exposed to fly infection for a period of 7 hours and then removed to damp chambers for incubation.

The weather was warm (about 15° C.), the sky cloudy, and the humidity high, a set of conditions that should have been favorable for the development of decay. Although 100 seed pieces were exposed to an abundance of flies, no decay was observed as a result.

Surface-sterilized tubers were broken open and exposed to flies of *Hylemyia cilicrura* in the fields under ideal conditions for rot as previously described. When several flies had settled and had walked

over the freshly opened potato surfaces, the tuber parts were closed and held together with the aid of rubber bands and placed in damp chambers. No decay resulted in the 45 tubers thus treated.

Adult insects were trapped in a freshly planted potato field and transferred to insect cages set over sterilized pans of soil in the laboratory. Figure 5 shows an insect cage of the type used. The eggs were removed by means of a fine camel's-hair brush to freshly cut potato slices in 2 sterilized Petri dishes. Approximately 10 eggs were used for each inoculated potato slice. The eggs hatched within a few hours, and the small maggots soon burrowed out of sight into the potato tissue. Rot developed in the potato slices inoculated in this manner. Several days later, potato slices in three additional Petri dishes were inoculated in a similar manner using a different lot

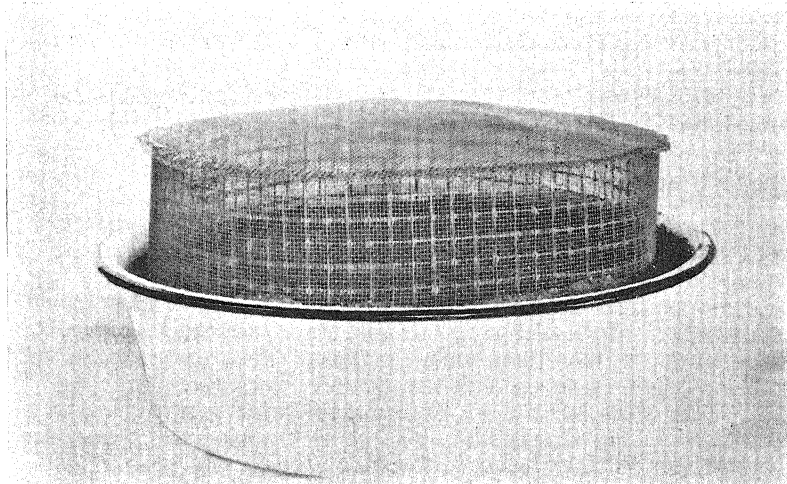


FIGURE 5.—Type of cage employed for inoculation studies using the seed-corn maggot. The adult insects were enclosed over pans of soil containing potato seed pieces.

of eggs. A rapid, soft rot also occurred in these potato slices. Toward the end of the planting season still another lot of potato slices was inoculated with eggs transferred from sterilized soil. This lot also was decayed by bacteria. No bacterial decay occurred in the uninoculated control potato slices. The results of these experiments are given in table 5 (lot 1).

In these inoculation experiments it was found desirable to keep the eggs quite moist. This was done by covering them with a bit of agar or with a moistened cover slip. The inoculations were also more successful if five or more eggs were used for each slice instead of a few or individual eggs.

Subinoculations of additional potato slices in moist chambers were made with the bacteria secured from these egg inoculations, with positive results. Pure cultures were also obtained of those found capable of causing blackleg. These cultures were identified by morphological and physiological characteristics as of the blackleg pathogen.

TABLE 5.—*Inoculation studies with the seed-corn maggot in Charleston, S. C.*

Lot No.	Sublots ¹	Stage of insect	Source of insect	Material inoculated	Type of decay
1	A-F	Egg	Cropped field.	Potato slices in sterilized Petri dishes.	White, soft.
2		Young larva	Rotted seed pieces from field.	Sterile potato slices in moist chambers.	Do.
3	{A-B	Adult	Cropped field.	Freshly cut seed pieces in unsterilized soil.	Do.
	{C	do	do	do	White, soft; also yellow.
4	{A	do	do	Freshly cut seed pieces in sterilized soil. ²	Rapid, yellow. ³
	{B-C	do	do	do	White, soft. ³

¹ 5 slices or seed pieces were used in each subplot, which was tested at a different time from the others in the same lot.

² The soil was sterilized with dry heat.

³ Rot developed in only 1 seed piece in each of the 3 pans.

Studies in Maine had shown that maggots taken from decaying potato seed pieces were capable of transmitting this decay to other seed pieces provided no protective cork layer had been formed. When maggots introduced the rot-producing organism into freshly cut seed pieces, an active decay resulted provided the soil was moist. Similar studies were made with the larval stages in South Carolina. Young maggots were removed from the interior of field-infected seed pieces, placed in freshly cut potato slices in moist chambers, and covered with moist, sterilized sand to prevent excessive drying. These inoculations resulted in an active decay (table 5, lot 2) which, when pricked into healthy potato sprouts, produced blackleg. Nine single-colony, pure-culture isolations were thus secured; all of the isolations belonged to the soft-rot group of bacteria.

The data show that the young larvae are more active and are better inoculating agents than the more mature maggots. As the maggots mature, they become sluggish and do not penetrate deeply into the potato tissue and thus are not very effective in causing the destruction of potato seed pieces. The maggots rarely, if ever, migrate to another seed piece after they have once penetrated a seed piece. This is especially true as they reach maturity. For this reason it is very unlikely that the maggots spread decay from one seed piece to another.

Additional experiments were made to determine more definitely to what extent larvae of the seed-corn maggot introduce pathogenic bacteria into healthy seed pieces.

In these experiments freshly cut surface-sterilized Irish Cobbler seed pieces were planted in pans of moist soil taken directly from the field, in comparison with similar seed pieces planted in sterilized soil. The pans were covered with insect cages, and adult insects were introduced as in the previous experiments. The freshly cut seed pieces planted in the unsterilized soil were severely attacked by the maggots resulting in decay (table 5, lot 3), which was particularly rapid and destructive when the soil was moist. The control seed pieces, which were allowed to suberize for 2 or 3 days and then planted in the unsterilized soil, remained entirely free from all maggot attack and bacterial decay. The factor of suberization will be discussed more fully later.

The injury from maggots and rot was greatly reduced in the studies with sterile soil, and only one seed piece in each of three pans of soil was injured (table 5, lot 4). None, or very few, primary lesions were present on the cut surfaces of the seed pieces in the sterile soil, and many of the young maggots perished for lack of suitable food. Apparently they had difficulty in attacking the healthy suberized potato tissue.

The decay organism was isolated in pure form from about the tunnels made by the feeding larvae. These cultures were similar to the soft-rot and yellow-rot organisms previously isolated from seed pieces taken from the field that were decayed or infected with maggots.

In view of the fact that the experiments (table 5, lot 4) were conducted under controlled conditions with the use of disinfected seed pieces and sterilized soil, the writer feels that it has been demonstrated that the adult of the seed-corn maggot is a carrier of the pathogenic bacteria. The results obtained in South Carolina studies support the data in the Maine studies.

INFLUENCE OF VARIOUS FACTORS ON INSECT INFESTATION AND DECAY OF POTATO SEED PIECES

INFLUENCE OF LESIONS CAUSED BY *PHOMA TUBEROSA*, FERTILIZER INJURY, AND DIFFERENT BACTERIA

The writer has noted that seed-piece decay by *Phoma tuberosa* and by *Fusarium* sp. is extremely common and that lesions caused by these fungi may contain pathogenic bacteria. The seed pieces affected in this manner generally are responsible for both missing hills and blackleg in abundance. Bacteria capable of producing blackleg often have been isolated from this source.

Seed-piece decay and blackleg were associated also with injury caused by fertilizer burning in the soil at planting time.⁶ The decayed seed pieces often contained numerous insects including the seed-corn maggot and the seed-potato maggot, as well as certain species of *Staphilinidae*, and the larvae of the larder beetle (*Dermestes lardarius*).

The fact that spotting by *Phoma tuberosa* and injury to the planted seed pieces by fertilizer were associated with the blackleg disease and with seed-piece decay, made it desirable to test the possibility of a relationship between these troubles and infestation by the seed-corn maggot.

FIELD STUDIES

Tests were conducted during 1930 in the field at Presque Isle, Maine. Seed pieces, infected recently with *Phoma* alone, injured by fertilizer, or affected with bacterial lesions on the cut surface, were exposed to attack by the larvae of *Hylemyia cilicrura* and *H. trichodactyla* under natural field conditions. The seed pieces affected with *Phoma* were selected from a bin of cut seed on Aroostook Farm. The fertilizer injury was induced by lightly sprinkling the cut surfaces of the pieces with commercial fertilizer before planting them in the soil. The seed pieces with bacterial lesions were secured from a bin of affected seed. The lots of seed were all from the same general Green Mountain stock and were grown in fields free from blackleg; the disease, therefore, could not have come from the parent stock.

⁶ Seed-piece injury caused by fertilizer burning appears to be less frequent since more modern planters have been developed.

The results are summarized in table 6. The data show certain relationships that may exist between the type of seed-piece spotting and the degree of attack by the seed-corn maggot.

TABLE 6.—*Insect injury and blackleg resulting from seed pieces affected with spotting by *Phoma tuberosa*, by bacteria, and by fertilizer injury, 1930*

Kind of seed	Treatment prior to planting	Seed pieces	Blackleg resulting from treatment	Prevalence of maggots in seed pieces
		Number	Percent	
With <i>Phoma</i> lesions.	Exposed to adult insects for 30 minutes in furrow.	115	0.0	None.
	Moistened and covered immediately.	100	.0	Do.
	Covered immediately	200	.0	Do.
Freshly cut.	do	200	.0	Do.
	Injured with fertilizer	200	1.5	Some maggots in all seed pieces.
Suberized.	do	100	2.0	None.
	Not injured with fertilizer	100	.0	Do.
With bacterial lesions.	Commercial storage; planted May 18, 1930.	100	62.0	Abundant.
	do	100	3.0	Maggots only in 3 seed pieces having decay and blackleg infection; no maggots in healthy seed pieces.
From same lot as preceding, but free from lesions.				
With bacterial lesions.	Commercial storage; planted May 22, 1930.	100	45.0	Abundant in all seed pieces showing decay.

The insects failed to attack the healthy seed pieces or those having only *Phoma* infection before planting. The bacterial lesions in contrast were very attractive to the insects, and each seed piece affected with bacterial spotting when planted later contained many maggots. Both the seed-corn maggot and seed-potato maggot were present in the affected seed pieces. Lesions induced by fertilizer injury also attracted the maggots. The degree of infestation following fertilizer injury was, however, much less than that resulting from infection by bacterial pathogens and the amount of blackleg was relatively small.

In 1930 an attempt was made to create a blackleg epidemic by injuring seed pieces with fertilizer. Seed pieces were dusted with fertilizer and covered with soil in the field. All of the seed pieces injured in this way became infested by *Hylemyia cilicrura*, whereas the uninjured controls were not. The maggots in each case entered through the tissue rendered soft by the toxic effect of the fertilizer.

The tests of 1930 were repeated during 1931 and 1932. The amount of blackleg resulting from these insect inoculations was very small, however, never being more than 1 or 2 percent. The writer does not feel that all of the blackleg that occurs in northeastern Maine can be attributed to inoculation by the seed-corn maggot. Other factors often determine the amount of infection.

In further tests, healthy potato tubers were cut in halves with a sharp knife and the freshly cut surfaces inoculated by streaking them with a soft-rot bacterial culture. The inoculated tubers were incubated in the laboratory for 1 day and then planted in the field. Each inoculated tuber part was alternated, when planted in the field, with a freshly cut, uninoculated control. In one experiment two plantings, each consisting of 100 inoculated tubers, were made, one on June 5 and the other on September 15.

The inoculated tubers and their control were examined for maggot injury at different intervals. No maggots were found in the uninocu-

lated controls, while 60 of the inoculated tubers planted on June 5 contained maggots, and 25 of those planted on September 15 were thus infested. The adults reared from the larvae in this experiment were of *Hylemyia trichodactyla*.

LABORATORY STUDIES

The role played by bacterial lesions in the degree of infestation of potato seed pieces by insects was tested further by experiments in the laboratory.

Irish Cobbler tubers free from bruises and blemishes were selected. Two notches in close proximity to each other were made with a sharp

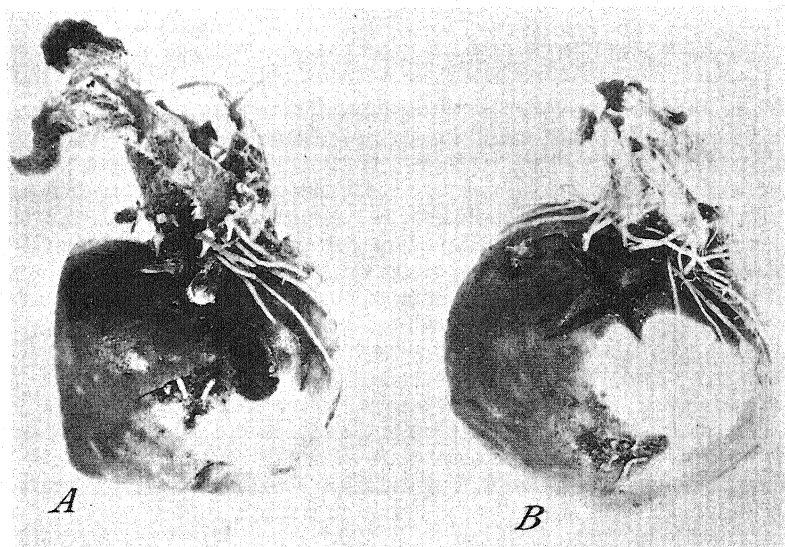


FIGURE 6.—A, Maggot attack in seed piece in area previously inoculated with a soft-rot culture; B, absence of attack in a similar seed piece that had not been contaminated with pathogenic bacteria. Both seed pieces were included in the same pan experiment in the presence of insects. Surface of seed pieces removed to show the extent of the decay.

knife through the periderm of the tuber. The freshly cut tissue of one of these notches in each tuber was inoculated with a soft-rot culture and the other notch was left uninoculated as a control. The tubers thus treated were planted in shallow pans of moist soil and kept in the laboratory.

Adult insects, trapped in the field, were brought to the laboratory and confined over the pans of soil in which seed pieces were planted. From 35 to 50 insects were used for each pan. Seven lots of seed each containing 10 seed pieces were subjected to insect attack.

The flies confined with the pans of potatoes deposited eggs in the moist soil in great numbers, and the young maggots began to hatch soon after the first day. The young larvae began to migrate in the soil in search of food and were found to enter the potato tubers only through the notches that had been inoculated with the soft-rot bacteria (fig. 6). This experiment shows that maggots are attracted by

bacterial decay and will migrate to such bacterial lesions. Under good growing conditions such small lesions would usually heal without destroying the seed piece; but when attacked by maggots the seed pieces are generally destroyed, and if a plant is formed it may develop blackleg.

Studies in Aroostook County, had led the writer to suspect that the source of seed-piece decay in the South was in the storage bin. Contrary to expectation, the unplanted seed lots observed in the Charleston area were exceptionally free from decay of all sorts, although information gained through conversation with growers would indicate that decay sometimes occurs. Very few decayed or injured seed pieces were found before planting, and the rots which followed after planting could hardly be attributed to bin contamination. This fact indicates that the problem of seed-piece decay and uneven stands as observed in the vicinity of Charleston, is somewhat different from that in Maine.

When healthy seed pieces were planted in soil from the Charleston area, it was noted that small bacterial lesions often developed on the cut surfaces and the seed pieces often contained numerous small maggots. Further field observations revealed the fact that the association of maggot injury with lesions was quite general and that this insect rarely attacked a firm and healthy seed piece.

A series of laboratory experiments were made in order to study this relationship. Ten seed pieces affected with small, superficial lesions were removed from the field and replanted in moist soil in pans in the laboratory along with seed pieces free from all evidence of decay. The healthy and the spotted seed pieces were alternated in the soil so as to compensate for any place effect. Approximately 30 flies captured in the field were enclosed with the pans and allowed to deposit their eggs in great numbers.

The pans were kept well moistened in subdued light at approximately 15° C. for 5 days before being examined. The results of this simple test were very striking. The eggs deposited by the flies had hatched in great numbers, and the young larvae had migrated to the seed pieces. The seed pieces affected by the shallow surface lesions had been greatly injured and in some cases were practically destroyed by maggot attack. The healthy seed pieces, in contrast, remained firm and bright and produced healthy vigorous sprouts. This experiment was repeated with similar results.

INFLUENCE OF SOIL BACTERIA

It was shown in the preceding experiment that maggots attacked seed pieces having superficial lesions that resulted when the potato sets were planted in the field under the conditions of Charleston. This indicated that the pathogenic soil organisms abundant in such soils might greatly influence the infestation of seed pieces by the seed-corn maggot. An experiment was made with the view of answering the question. Freshly cut seed pieces and suberized controls were planted both in sterilized soil and in soil taken directly from the field. Adult insects captured in wire-screen traps in the field were confined with the planted seed in the two kinds of soil. The soil was kept well watered and at a temperature of approximately 15° C. for the duration of the test. Each treatment was made in

duplicate, and examinations of the seed pieces were made at different intervals.

TABLE 7.—Seed-piece decay and maggot injury in sterilized and unsterilized soil ¹

Lot No.	Treatment of seed	Adult insects added ²	Seed-piece decay and insect injury—	
			In sterilized soil	In unsterilized soil
		Number		
1	Freshly cut	0	None	Surface lesions on all seed pieces.
2	Suberized ³	0	do	None.
3	Freshly cut	0	do	All seed pieces badly decayed.
4	do	30-35	do	Severe insect injury in all seed pieces; complete decay.
5	do	30-35	3 seed pieces were penetrated by maggots; 2 of these developed rot. ⁴	Do.
6	Suberized ³	30-35	None	None.

¹ Each lot contained 30 seed pieces. The soil was sterilized by dry heat.

² Adult insects trapped in the field removed to the laboratory. The adults deposited eggs in great numbers in the soil. The eggs hatched within 12 to 24 hours and immediately migrated to favorable sources of food.

³ Suberized by being placed in moist chambers for a period of 5 days.

⁴ The maggots attacked the seed pieces slightly on the cut surfaces. Each rotted seed piece contained approximately 20 small maggots, all of which had entered at 1 small hole, probably originally a small lesion. The soft rot organism (*Erwinia carotovora*) was isolated from this material, demonstrating further that the seed-corn maggot may introduce pathogenic bacteria into potato seed pieces.

The results of this experiment are summarized in table 7. Seed pieces previously suberized for 5 days in moist chambers at 15° C. remained healthy and free from all insect injury in both sterilized and unsterilized soil; also, no lesions developed on freshly cut seed pieces planted in the sterilized soil free from insects. On the other hand, the freshly cut, unsuberized seed pieces planted in unsterilized soil all developed decay in various degrees of severity. The severity of the decay apparently depended on the amount of soil moisture present. The injury by the seed-corn maggot was also very extensive.

Freshly cut seed pieces planted in sterilized soil were not spotted and escaped attack by insects. However, the maggots penetrated three seed pieces apparently free from externally obvious decay. Two of the seed pieces thus attacked later developed an internal bacterial soft rot, with only a very small opening to the outside. The maggots apparently had entered in quite large numbers through a relatively small opening caused by bacteria introduced by the adult insect on the eggs.

The seed pieces planted in sterilized soil that were attacked by the seed-corn maggot yielded pathogenic bacteria, later identified as belonging to the blackleg or soft-rot group. Since the soil and the seed pieces were sterilized before planting, there can be no doubt that the organisms were introduced by the insects.

This experiment shows that the potato soils in the vicinity of Charleston contain pathogenic bacteria that are capable of injuring the planted potato sets. These soil bacteria often determine the success of the attack on potato seed pieces by the seed-corn maggot. The fact that soft-rot bacteria were isolated from seed pieces planted in sterilized soil is also significant. It shows that pathogenic bacteria may in some cases be introduced by the seed-corn maggot.

An experiment was also made which showed that the lesions resulting in the field and those produced in the laboratory were similar. Both were caused by the soft-rot bacteria (*Erwinia carotovora*) and by

an unnamed bacterium possessing polar flagella and capable of causing a yellow rot in potato seed pieces.

In the fields about Charleston the lesions result from infection originating in the soil. The larvae of *Hylemyia cilicrura* enters the seed pieces through these superficial lesions and penetrate deeply into the tissues, preventing the tubers from healing and hastening the decay. In Maine both the seed-corn maggot and the seed-potato maggot are attracted by bacterial lesions. Primary infection in Maine, however, generally occurs before the seed is planted in the soil.

EFFECT OF EXCESSIVE EARLY DRYING OF SEED PIECES

FIELD STUDIES

In another series of experiments it had been noted that missing hills and seed-piece decay were correlated with injury of the cut seed by excessive early drying prior to planting. The injury consists of a drying and killing of the surface layers of cells because an adequately protective new cork layer failed to form. It was not known, however, whether this drying increased the amount of infestation by insects. In order to gain this desired information, seed pieces injured by such drying were exposed to maggots in the field in comparison with healthy controls and the relative degree of injury was noted.

The Irish Cobbler variety was used. Whole tubers that were free from all evidence of disease and bruises were each cut into 4 seed pieces of equal size, 2 of these were exposed for 1 day in the bright sun and drying wind, while the other 2 were stored in a cool basement until planted. The seed pieces exposed out of doors became somewhat shriveled and darkened on the cut surfaces while those stored in the basement remained bright and firm. The 2 lots of seed were alternated in the row when planted in the field. Each lot consisted of 100 seed pieces, and planting was made on three different days, May 20, June 5, and June 10. The lots were examined at different times during the season and the degree of injury by insects was noted. The decay that developed was 10, 55, and 30 percent, respectively. No maggot infestation occurred in the seed pieces planted May 20, whereas the maggot infestation of the planting on June 5 was 40 percent that of June 10, 28 percent. No decay or maggot infestation was observed in the controls.

Early exposure to sunlight and dry air therefore may result in a high percentage of maggot infestation and decay in the planted seed pieces. This generally results in poor stands. Plants that survive such treatment may be small and weak and yield poorly. Many of the uneven stands found in the potato-growing areas occur because of this type of injury. In no case in this experiment was a firm and healthy seed piece attacked by these insects. The insects in these tests were mostly *Hylemyia trichodactyla* Rond. Bushnell (4) pointed out that poor potato stands in Ohio may result from injury to cut surfaces from exposure to sun and wind and reported that an exposure of more than half an hour greatly reduced the percentage of plants that emerged from the soil. He does not mention whether the practice results in an increase of insect infestation.

LABORATORY STUDIES

The effect of drying and sunlight on the amount of seed-piece decay and maggot infestation was tested also in the laboratory. Potato seed pieces were injured by exposure to direct sunlight and dry air. Five seed pieces thus injured were planted in pans of soil with five healthy freshly cut seed pieces, making 10 in each pan. Flies were introduced from the field and were confined over the pans. The seed pieces were examined after the maggots had developed and begun to feed. Seven lots of seed pieces were used.

The data obtained support the conclusion that early exposure of freshly cut seed pieces to sunlight and dry air favors attack by the

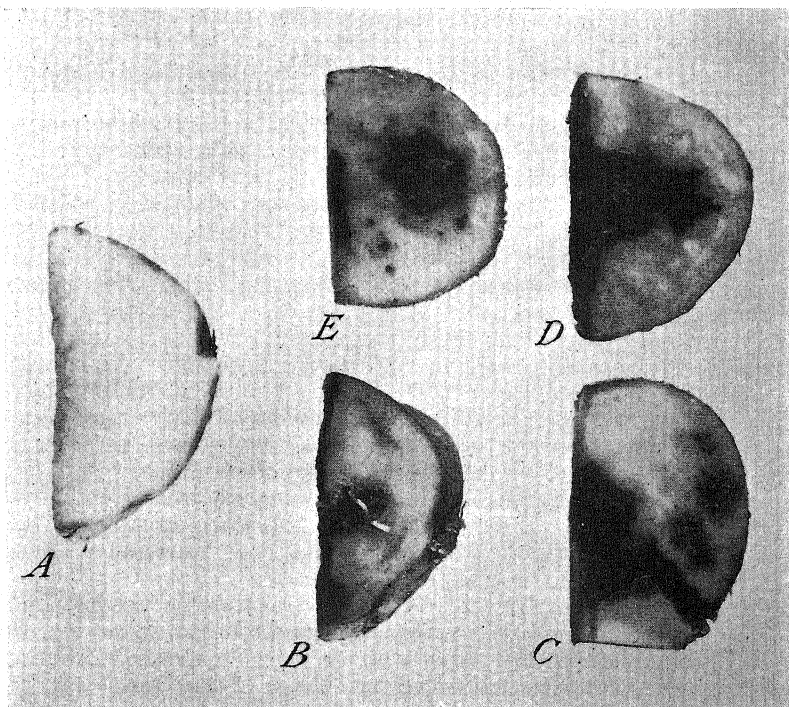


FIGURE 7.—Maggot injury resulting in the field in seed pieces that had been injured by exposure to dry air before being planted: A, Control seed piece that was not injured by drying and was not attacked by the maggots; B-E, injured seed pieces. Note the maggots in B.

maggot. No insects were found in the healthy seed pieces. The injured seed pieces, on the other hand, were in the majority of cases badly decayed and were infested with larvae of both *Hylemyia cili-crura* and *H. trichodactyla*. Figure 7 shows maggot attack on seed pieces injured by excessive drying. In these tests the seed-potato maggot was most commonly encountered although the seed-corn maggot also was present.

INFLUENCE OF SOIL MOISTURE

The initiation of seed-piece spotting and decay is influenced by the amount of soil moisture. This fact suggested that decay in seed pieces following infestation with maggots might be influenced in a similar manner. The writer, in his isolations from seed pieces injured by maggots, was often able to secure pathogenic bacteria from the region about the wounds made by the seed-corn maggot in spite of the fact that no decay was obvious. It would seem that these latent bacterial infections should cause a more rapid decay under the right environmental conditions.

Leach (14) has shown that tubers held under moist conditions are unable to form a protective wound-cork layer. According to his data (14, p. 224) the tubers were only slightly suberized when the moisture content in sand was from 12.03 to 12.59 percent (of dry weight) and the protective layer failed to develop entirely when the soil moisture was 14.75 percent.

Freshly cut seed pieces were subjected to attack by young maggots in the laboratory. After maggots had penetrated and burrowed into them, the seed pieces were removed from the pans of soil and divided into two similar lots. One lot thus injured was planted in relatively dry, and the other in relatively moist soil. In the second series, freshly cut seed pieces, free from maggots, were planted in a similar manner. A third set of pans contained suberized seed pieces planted like those in the first two sets. Adult flies from the field were introduced into cages over the freshly cut and suberized seed pieces. Twenty seed pieces were used in each test. Each set of comparisons was repeated.

The seed previously injured by the burrowing of the young maggots was completely destroyed when subjected to the conditions in the moist soil. The same type of injured seed pieces planted in the drier soils developed no apparent decay and sprouted normally. Cultural isolation showed that these seed pieces had been inoculated with pathogenic bacteria but that their growth and advancement into the potato tissue had been checked.

Microscopic examination of freehand sections of the potato tissues subjected to conditions in the moist soil revealed that no wound cork had been formed about the insect wounds. In the dry soil the insect wounds of seed pieces were blocked by a layer of cork tissue.

Leach (14, p. 224) showed that a protective layer failed to develop when the moisture content of the soil was 14.75 percent. The dry soil used in the experiments reported here had approximately 6 to 7 percent of moisture and the wetter soil approximately 9 to 10 percent. In no case was the moisture excessive or the soil completely waterlogged and the conditions approximated quite closely those that often exist under field conditions.

The freshly-cut seed pieces planted with flies developed no rot or insect injury in dry soil. In wet soil, however, these unsuberized seed pieces were completely destroyed by a bacterial decay.

The experiments show that when such infested seed pieces are subjected to sufficient soil moisture, the decay becomes rapid and may totally destroy the potato sets. High soil moistures often occur after heavy rains. If unsuberized seed pieces are planted under such conditions they quickly develop the necessary soil lesions for insect attack.

The insects which attack the seed pieces through the lesions may introduce additional pathogenic microorganisms and under the favorable conditions of high soil moisture the seed pieces are rapidly destroyed.

The suberized seed pieces planted in both moist and dry soils developed no decay, nor were they injured by the insects. The seed pieces sprouted normally and produced healthy plants.

RELATION OF WOUND-CORK FORMATION TO ATTACK BY THE SEED-CORN MAGGOT

Reid, Peacock, and Wright found that (17) in the trucking area in the vicinity of Charleston, S. C., the injury to planted potato sets caused by the seed-corn maggot could be controlled by proper suberization of the seed before planting. They recommend that seed stock be thoroughly disinfected before cutting and allowed to cork over for a period of 10 days or 2 weeks at a temperature of 55° to 65° F. at a relative humidity of 80 to 90 percent before being planted. Johnson (10), however, stated that results in Virginia would seem to justify cutting and planting within a few hours. The growers in his section follow various practices—some cut their seed 2 or 3 days before planting, some few cut the tubers a week before planting, while others cut and plant immediately. Johnson has depicted a field with an exceedingly poor stand which resulted from planting tubers that had been cut and held in storage for 10 days (10, *fig. 33*) and in contrast an excellent stand which resulted from planting freshly cut seed pieces (10, *fig. 34*).

According to Reid et al. (17) in the spring of 1921, when the weather was unfavorable for rapid planting along the entire Atlantic seaboard, the injury from attacks of the seed-corn maggot was unusually severe, and as much as 50 percent of the planting in the commercial production areas of the Carolinas was destroyed. That large losses still occur along the Atlantic seaboard because of seed-piece decay can be seen by consulting the more recent market news service records.

As pointed out elsewhere in this paper, in the Charleston truck-crop area freshly cut potato seed pieces often develop small surface lesions when planted, if the soil is moist and unsterilized. It has been suggested that suberization is an aid in preventing the formation of these primary lesions which are quite essential for the attack by the seed-corn maggot.

During the seasons of 1929 and 1930, the writer attempted to imitate, under laboratory conditions, the planting practice of the growers in South Carolina. Freshly cut seed pieces as well as suberized seed pieces were exposed to infection by soil organisms and to attack by the seed-corn maggot. The pans of soil were kept fairly moist and warm so as to be ideal for the germination of the potato sets.

Freshly cut seed pieces in about 24 hours after were examined and planting found to have numerous exceedingly small decayed areas that appeared to be only a few cells in depth and from 1 to several millimeters in diameter. All unsuberized seed pieces planted in unsterilized soil developed these primary lesions in abundance on the cut surfaces, whereas the suberized seed pieces did not. Figure 8 shows these primary lesions.

The seed pieces with the primary soil lesions were replanted in pans of unsterilized field soil in the laboratory. The infected seed

was alternated with seed pieces which had originally come from similar seed stock but which had been allowed to cork over for a period of 4 days in moist chambers. Approximately 30 flies of the seed-corn maggot were taken directly from the field and enclosed by a wire screen with the planted seed pieces.

After a 4-day period the seed pieces were examined. The maggots in great numbers had penetrated the seed pieces affected with the lesions. The corked-over seed pieces, on the other hand, were entirely free from maggot attack. Figure 9 illustrates the maggot injury on the unsterilized seed pieces.

The experiment was repeated with a few variations. Three pans of inoculated seed pieces were used in the second experiment. In

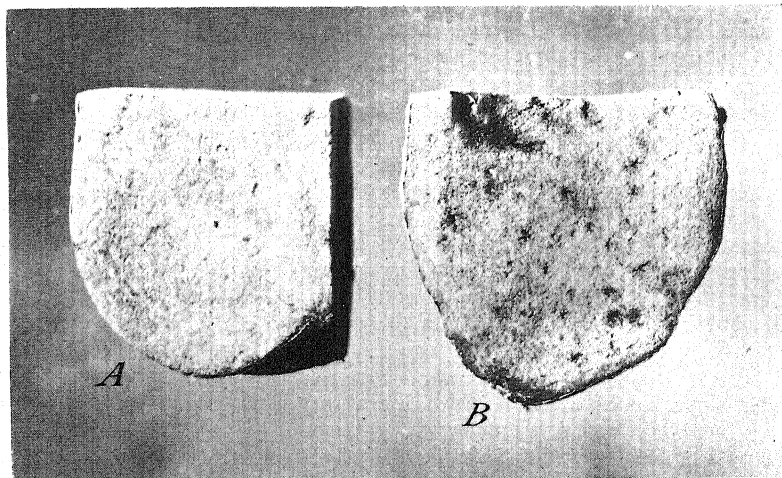


FIGURE 8.—Primary bacterial lesions formed on freshly cut seed piece planted in unsterilized soil from Charleston, S. C.: *A*, Suberized seed piece free from bacterial infection; *B*, unsterilized seed piece with numerous lesions caused by pathogenic soil bacteria. The maggots enter the seed pieces through these superficial lesions.

one pan of unsterilized field soil, 30 to 35 flies were confined with seed pieces having lesions in comparison with seed suberized for only 2 days; in another pan, 20 to 25 flies were confined with seed pieces having lesions in comparison with seed suberized for 4 days; and in the third pan seed pieces that had been suberized for 4 days were compared with spotted seed pieces. No flies were included in the third pan.

The pans were placed in the laboratory at a temperature of approximately 15° C. for the duration of the test. The seed pieces were examined daily and observations made regarding the entrance by maggots.

The larvae began to emerge from the eggs deposited in the soil after 1 day and immediately entered the seed pieces through the lesions induced by soil infection. Entrance was made only through the areas where the potato surface had previously been injured by the pathogenic soil bacteria. The young maggots apparently avoided

all the surface tissue that had a protective cork layer. The seed previously suberized remained entirely free from all decay and produced healthy sprouts. The seed pieces attacked by insects were practically destroyed within 6 days. The seed pieces infected by soil organisms and with no insects present continued to decay. The rot was not rapid, however, for extensive and healthy sprouts developed.

The results of this experiment would indicate that the young larvae of the seed-corn maggot, after having been attracted to the lesions resulting from soil infection, by their tunneling and feeding activities aggravate the infected areas and prevent the natural development of

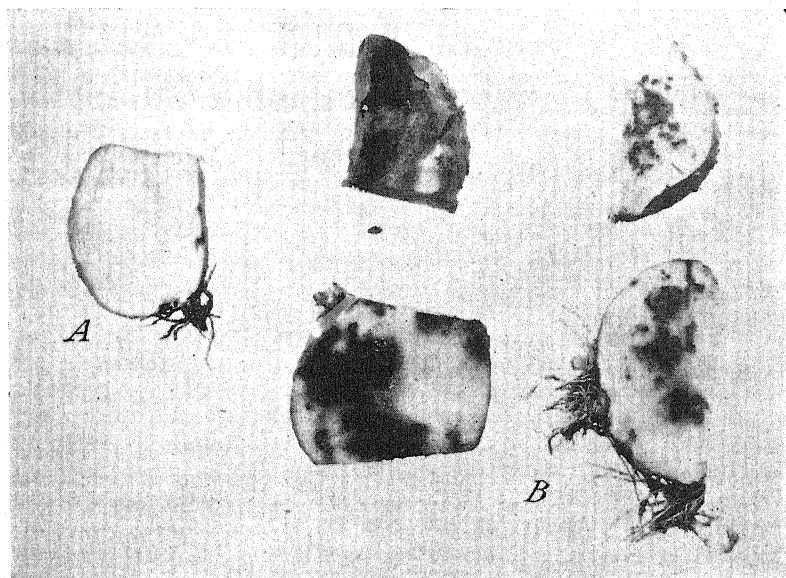


FIGURE 9.—Injury by the seed-corn maggot on unsuberized seed pieces infected from soil containing pathogenic bacteria. The young maggots penetrated the seed pieces through primary lesions similar to those shown in figure 8: A, Suberized seed pieces not injured by maggots; B, unsuberized and rotted seed pieces with maggot injury. The suberized and the unsuberized, rotted seed pieces were planted in the same pans of soil with the adult insects.

a protective wound periderm, the result being that the seed pieces are destroyed by the pathogenic bacteria.

These studies were continued in South Carolina during the season of 1930 under very similar conditions.

The results of these experiments as well as the general plan of the tests are summarized in table 8. Lesions developed where freshly-cut potato tissue was exposed to moist soil (table 8, lot 1); maggots entered the unsuberized, decayed seed pieces in great numbers, the seed pieces being practically destroyed by their action and by the bacterial decay thus aggravated (table 8, lots 2 and 3). The suberized seed pieces in all cases remained free from decay or insect injury and developed large healthy sprouts. The infested seed pieces, on the other hand, generally failed to germinate.

TABLE 8.—Comparison of injury by maggots and the extent of decay occurring in suberized and freshly cut potato seed pieces ¹

Lot No.	Kind of seed piece	Adult flies added ²	Extent of insect injury	Controls ³
1	Freshly cut ⁴	Number 0	Suberized.
2	do.....	50	Complete destruction.....	Do.
3	do.....	40	Severe, followed by decay.....	Do.
4	Suberized; 1 surface removed with knife.....	40	Severe. All injury on freshly cut surface.	Suberized; not cut.
5	Suberized; cork layer broken by freshly cut notches.	25	Maggots entered 2 seed pieces through notches, resulting in decay.	Suberized; not notched.
6	Freshly cut.....	25	Severe, followed by decay.....	Suberized.

¹ This experiment was conducted in the laboratory. Each test consisted of 12 seed pieces in addition to 5 controls.

² The flies were trapped in the field and transferred to cages covering the pan experiment in the laboratory. The flies deposited their eggs in the soil and the young larvae generally hatched within 12 to 24 hours.

³ No surface lesions or insect injury in any series.

⁴ Numerous surface lesions formed whenever a freshly cut surface came into contact with unsterilized soil.

The importance of suberization to the failure of penetration by the seed-corn maggot was further shown by the data. The surface from one side of the suberized seed pieces was removed and the young maggots migrated to these freshly cut surfaces and entered through the small bacterial lesions that developed there from soil contamination.

In one series (table 8, lot 5) notches were cut with a knife through the suberized surfaces just before planting the seed pieces in moist soil. The small maggots migrated to these injured seed pieces and entered through the notched area where bacteria had initiated rot in small places. The notched seed pieces were found to contain, in some cases, approximately 100 of the insect larvae, and the process of destruction was rapid. In no case was a properly suberized and uncut seed piece injured by maggots or by the rotting soil microorganisms.

These studies show that proper healing of the potato seed pieces eliminates seed-piece spotting caused by soil infection, and this in turn affords a partial control measure for the injury caused by maggots under Charleston conditions.

LABORATORY STUDIES WITH INSECTS OTHER THAN THE ANTHOMYIIDS

In addition to the anthomyiids, discussed in this paper, some of the other insects found commonly associated with potato rots were tested on healthy potato tubers to determine whether they also were capable of introducing the soft-rot organisms and thus could be responsible for the infection that often occurs on seed pieces. The insects tested were the common housefly (*Musca domestica*), the false crane fly (*Trichocera*, sp.), two pomace flies (*Drosophila funebris* Fab. and *D. bruschii* Cog.), the fungus gnat (*Sciara tridentata*), the larder beetle (*Dermestes lardarius*), the wireworm (*Cryptohypnus abbreviata*), and a staphylinid insect.

The tests were made in glass moist chambers located in a cool laboratory basement. The insects in each case were taken directly from the infective material and transferred to the tubers included in

the inoculation tests. In each case many insects were available, and the conditions for rot were ideal so that any failure to secure infection cannot be attributed to the use of too few insect vectors or to unsuitable environmental conditions. With one exception, the insects used in these studies did not initiate the infection or aggravate the bacterial decay of potato seed pieces. The common housefly (*Musca domestica*), the false crane fly (*Trichocera* sp.), the pomace flies (*Drosophila*), and the fungus gnat (*Sciara tridentata*),⁷ although commonly associated with decaying potatoes, apparently act only as scavengers and feed on the decaying organic matter. All of these insects can live and reproduce on decaying potato tissue. The larvae do not, however, penetrate into the healthy part of the potato tuber but remain in those portions that have disintegrated. These insects failed to develop, and in most cases died, when placed in the presence of only healthy potato slices. When decayed tubers were added they developed and reproduced normally.

The larvae of the larder beetle (*Dermestes lardarius*) caused a slight injury to the healthy potato slices in some cases. This injury was quite superficial, however, and no active decay resulted. This insect, in both adult and larval stages, is commonly found feeding on the decayed tissues associated with potato blackleg. It apparently does not disseminate the pathogen of blackleg and seed-piece decay. The larvae of the wireworm (*Cryptohypnus abbreviatus*) readily penetrated the healthy potato slices in these experiments, but no decay was noted as a result of this injury.

The staphylinid was the only kind of insect used in these studies which appeared capable of causing the destruction of potato seed pieces. The larvae of this insect are very active and burrow deeply and beyond the decayed area, in contrast with most of the other insects found in association with seed-piece decay which appear to be merely feeding on the decayed material resulting from the infection. Staphylinids spread the infection by carrying the infective material through the protective cork layer laid down by the potato seed piece. The writer in other experiments has inoculated healthy seed pieces with soft-rot bacteria by means of staphylinid larvae. Pure cultures of the bacterium isolated from seed pieces attacked by this insect have been studied in some detail. The physiological, morphological, and pathological characteristics of this organism place it among the soft-rot group of bacteria.

Observations for several seasons have shown that this staphylinid is exceedingly common in decayed potato material in the field. It is generally present in plants affected with blackleg or seed pieces infected with bacterial or fungus organisms. The writer has found the larvae burrowing in potato stems and seed pieces infected with blackleg. The adults are often seen feeding on infected potato seed pieces.

In no case under field conditions have staphylinids been found to attack seed pieces that had been properly suberized before being planted. Lesions occurring on the cut surfaces of the seed pieces when planted act as avenues of attack by staphylinids.

⁷ These insect determinations were made by the late Dr. C. R. Phipps, at that time entomologist of the Maine Agricultural Experiment Station.

FIELD OBSERVATIONS ON INSECTS THAT COMMONLY ATTACK
POTATO SEED PIECES IN AROOSTOOK COUNTY

When freshly cut seed pieces are exposed in the field, soft rots often develop. From such rotted seed pieces the writer has reared a number of dipterous insects. Among these are the common housefly (*Musca domestica*), the seed-potato maggot (*Hylemyia trichodactyla* Rond.), the seed-corn maggot (*H. cilicrura* Rond.), and two pomace flies (*Drosophila funebris* Fab. and *D. bruschii* Cog.).⁸ In such decayed potato material a histerid beetle also has been commonly observed.

In Aroostook County very often planted seed pieces are injured by a wireworm (*Cryptohypnus abbreviatus* Say.). In 1928 and 1929 approximately 25 percent of the seed pieces in one experiment were attacked by this insect. During 1930 the writer examined approximately 2,000 seed pieces of which 40 percent had been injured by wireworms. Although a high proportion of the seed pieces had been injured by the burrowing of these insects, the economic loss appeared to be quite negligible and the seed pieces germinated well. *C. abbreviatus* apparently attacks healthy seed pieces and has never been observed by the writer to be a carrier of bacterial decay, although in a few instances a slight fungus decay has been noted about the tunnels. This species of wireworm attacks healthy seed pieces in contrast with the habits of some other insects common in potato fields which are apparently attracted to decaying organic matter. The writer feels that this particular species of wireworm does not play an important role in the dissemination of blackleg and seed-piece decay.

The writer has examined numerous decayed potato seed pieces in the field. During 1927 and 1928 practically all the decayed seed pieces in fields showing blackleg were infested by the seed-corn maggot or the potato-seed maggot. In these same fields the healthy seed pieces were free from maggot attack. During the season of 1932, approximately 2,000 individual seed pieces were examined; only 2 showed bacterial decay, and each of these contained approximately 40 larvae of the seed-corn maggot. The writer has never noted healthy potato seed pieces infested by either of these insect larvae. The seed-corn maggot is generally present, however, when the seed pieces contain a bacterial decay and are exposed to the conditions of the soil in the field.

Adult and larval stages of the larder beetle (*Dermestes lardarius*) are also very prevalent about decaying seed pieces and plants affected with blackleg.

Staphylinids are often abundant wherever decaying potato tubers are present in the soil. Observations have shown that these insects are probably the most abundant and appear to have some relation to seed-piece decay and blackleg. The larvae and also the adults are very active feeders and are found burrowing into seed pieces and plants affected with blackleg.

SUMMARY

The studies presented in this paper were conducted to determine the part taken by certain insects in the dissemination of blackleg and

⁸ These insect determinations were made by the late Dr. C. R. Phipps, at that time entomologist of the Maine Agricultural Experiment Station.

seed-piece decay in the potato, the factors which influence the attack by insects, and the consequent development of these diseases.

The seed-corn maggot (*Hylemyia cilicrura* Rond.), a common insect in Maine, may injure planted potato seed pieces. The life history and the habits of this insect were studied.

The seed-potato maggot (*Hylemyia trichodactyla* Rond.), also attacks potato seed pieces in Maine. The data indicate that this insect has a more specific preference for potato tissue in Maine than does *H. cilicrura*. Its habits and life history are similar to those of *H. cilicrura*.

Soft rot and other pathogenic bacteria are intimately associated with the different stages in the development of the seed-corn maggot both in Maine and South Carolina and were isolated from the surface of eggs and from within the puparia and the adult. Bacteria capable of producing blackleg and decay in potatoes were isolated from puparia of the seed-corn maggot that had over-wintered in potato fields of Aroostook County, Maine.

The soft-rot organism appeared to aid in the development of the seed-corn maggot when present on agar media in which they were feeding. The presence of other soil organisms was, however, much more important in promoting a rapid growth of this insect.

Blackleg and seed-piece decay were produced in Maine under laboratory conditions by inoculations with the seed-corn maggot and the seed-potato maggot. The seed-corn maggot also successfully inoculated potato seed pieces and slices with *Erwinia carotovora* and other pathogenic bacteria under controlled conditions in the laboratory in South Carolina. The adult insect of the seed-corn maggot did not successfully inoculate potato seed pieces with pathogenic bacteria by mere contact under field conditions in South Carolina. These insects were not found in potato bins.

Field and laboratory studies show that these insects will not attack seed pieces that are free from decay. They generally are attracted to bacterial lesions on seed pieces, or to seed pieces that have been injured by fertilizer burning or by desiccation. The maggots were not attracted to lesions which were caused by *Phoma tuberosa* and other fungi and which were free from bacteria.

Shallow surface lesions are formed when unsuberized seed pieces are planted in moist, warm soil in South Carolina. The young contaminated larvae of the seed-corn maggot migrate and enter the seed pieces through these lesions. The insects aggravate the decay by their burrowing, and the seed pieces are completely destroyed if the soil moisture content is relatively high.

Suberizing the cut seed prevents the formation of the primary lesions through which the maggots enter and this eliminates the injury caused by the insects in the South.

In Aroostook County seed pieces do not normally develop surface lesions as a result of soil contamination such as occurs in the South, and the attack by the seed-corn maggot and the seed-potato maggot occurs through lesions on potatoes in the bin prior to their being planted.

Freshly cut potato seed may be safely planted in Aroostook County without danger of injury by maggots.

Freshly cut seed pieces when injured by excessive drying often develop a softened condition on their cut surfaces, and bacterial con-

tamination may become established there. The seed-corn maggot and the seed-potato maggot attack the seed pieces through these diseased areas.

Seed pieces that have been injured by maggots may decay rapidly provided the soil is sufficiently wet.

Certain other insects are commonly associated with decaying potato parts in the field and in the storage bins of Maine but apparently do not have a part in bringing about these conditions. Among these insects are the common housefly (*Musca domestica*), the false cranefly (*Trichocera* sp.), two pomace flies (*Drosophila funebris* Fab. and *D. bruschii* Cog.), the fungus gnat (*Sciara tridentata*), and the larder beetle (*Dermestes lardarius*).

The wireworm larvae (*Cryptohypnus abbreviatus*) often attack potato seed pieces in northern Maine, but their presence has not been associated with a bacterial decay.

A certain staphylinid was found associated with decaying seed pieces and with plants affected with blackleg. This insect may aid in the dissemination and the destruction of potato seed pieces in Maine.

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SUSCEPTIBILITY OF SPECIES OF CUPRESSACEAE TO CROWN GALL AS DETERMINED BY ARTIFICIAL INOCULATION¹

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INTRODUCTION

The natural occurrence of crown gall on conifers has been reported by Persons (2)² and by Barrus, Boyd, and Wood³ on *Juniperus sabina*; by Brown and Evans (1) on *Cupressus arizonica*; by Smith (5) on *Libocedrus decurrens*; by Metcalf,⁴ who observed large galls at and below the ground level on a street tree of *Araucaria bidwillii*; and by Rounds,⁵ who found a gall 10 inches in diameter on *Araucaria excelsa*.

Crown gall has been artificially produced on *Sequoia gigantea* and *S. sempervirens* (3), on *Araucaria bidwillii* (4), *Libocedrus decurrens* (5), *Cupressus* spp. (7), and on *Taxus baccata* var. *erecta* (6).

The results of artificial inoculations on species of Cupressaceae with *Bacterium tumefaciens* Smith and Townsend (*Phytomonas tumefaciens* (Smith and Town.) Bergey et al.) are reported in this paper. The cultures of the organism used in the inoculations were isolated from *Prunus persica*, *Libocedrus decurrens*, and *Salix* sp.

MATERIALS AND METHODS

The Cupressaceae under investigation consisted of species of *Cupressus*, *Juniperus*, *Libocedrus*, *Thuja*, *Thujopsis*, and *Chamaecyparis*.

The plants used in this experiment were: (1) Small seedlings obtained from the Rancho Santa Ana Botanic Garden; (2) plants from the United States Department of Agriculture carrying the plant introduction numbers; (3) small trees purchased from a local nursery; (4) rooted cuttings from certain species; (5) plants grown from seed at the University of California Citrus Experiment Station; and (6) large trees growing on the campus. The sources of the plants are shown in table 1. Seedlings and small trees were grown in 5-gallon tin containers in a lath house during the experiment and were in a vigorous growing condition.

Inoculations were through wounds made by a steel needle or through deeper wounds made by cutting through the inoculum into the tissue with a scalpel. The wounds were left unprotected or were protected in various ways. In some tests the wounds were wrapped with paraffin paper. Desiccation in other tests was further checked by tying moist cotton over the wounds, which were then wrapped with paraffin paper fastened at the ends to the plant with nurseryman's

¹ Received for publication June 5, 1939. Paper No. 404, University of California Citrus Experiment Station.

² Italic numbers in parentheses refer to Literature Cited, p. 925.

³ BARRUS, M. F., BOYD, O. C., and WOOD, JESSIE I. DISEASES OF PLANTS IN THE UNITED STATES IN 1930. U. S. Bur. Plant Indus. Plant Dis. Rptr. Sup. 81: 133. 1931. [Mimeographed.]

⁴ Letter dated March 5, 1938, to the author from Woodbridge Metcalf, extension forester, University of California.

⁵ Oral report to the author July 8, 1939, by M. B. Rounds, Associate in the Citrus Experiment Station.

tape. When inoculations were made near the base of the tree, a waterproof plant pot was placed around the inoculated trunk and filled with fine gravel which was kept moist. A few inoculations were made on the trunk at and below the ground level. Some of the smaller plants were inoculated and kept for a time in a moist chamber, and galls were frequently developed by the use of this method when results from unprotected inoculation wounds had failed. Positive results were obtained from all of these methods on susceptible hosts.

TABLE 1.—Summary of artificial inoculations through wounds with *Bacterium tumefaciens* on species of *Cupressaceae*

Host species	Sources of plant material ¹	Inoculations	Galls ²	Gall diameter range ²
		Number	Number	Millimeters
<i>Cupressus arizonica</i> Greene.....	Seedlings.....	93	18	5-20
Do.....	S 2357, S 2358.....	35	7	5-15
<i>Cupressus bakeri</i> Jeps.....	S 2127, S 2131.....	30	4	3-30
<i>Cupressus benthami</i> Carr.....	SPI 114036.....	15	7	5-20
<i>Cupressus duttonii</i> Jeps.....	S 2157.....	15	7	3-25
<i>Cupressus forbesii</i> Jeps.....	S 2315, S 2319, S 2335.....	50	9	4-20
<i>Cupressus glabra</i> Sudw.....	SPI 112084.....	20	0	0
Do.....	C. E. S., Nursery.....	155	0	0
<i>Cupressus guadalupensis</i> Wats.....	S 2069.....	35	0	0
<i>Cupressus goveniana</i> Gord.....	S 2181, S 2182.....	30	2	3-20
<i>Cupressus lusitana</i> Mill.....	SPI 73844.....	60	5	10-25
<i>Cupressus knightiana</i> Mast.....	Nursery.....	10	3	2-3
<i>Cupressus macrocarpa</i> Hartw.....	S 2177, S 2184.....	25	7	3-35
Do.....	Seedlings.....	165	3	2-20
<i>Cupressus macnabiana</i> Murr.....	S 2118, S 2154.....	25	2	2-20
<i>Cupressus montana</i> Wigs.....	S 2067.....	30	0	(³)
<i>Cupressus nevadensis</i> Abrams.....	S 2159.....	15	3	3-5
<i>Cupressus pygmaea</i> Sarg.....	S 2133, S 2137.....	30	7	6-35
<i>Cupressus sargentii</i> Jeps.....	S 2156, S 2149, S 2185.....	43	10	12-35
<i>Cupressus sempervirens</i> L.....	C. E. S.....	45	9	10-20
<i>Cupressus thurifera</i> Schlecht.....	S 2356.....	28	4	5-25
<i>Cupressus torulosa</i> Don.....	SPI 112085.....	15	4	3-10
<i>Thuja plicata</i> Don.....	Nursery.....	45	9	10-20
<i>Thuja occidentalis</i> L.....	do.....	100	5	5-25
<i>Thuja orientalis</i> L.....	C. E. S.....	30	17	10-30
<i>Thujaopsis dolabrata</i> Sieb. and Zucc.....	Nursery.....	45	2	10-15
<i>Chamaecyparis lawsoniana</i> Parl.....	do.....	90	2	0
<i>Libocedrus decurrens</i> Torr.....	do.....	95	34	10-30
<i>Juniperus ashei</i> Buchholz.....	SPI 124965.....	6	3	(³)
<i>Juniperus californica</i> Carr.....	C. E. S.....	95	0	0
<i>Juniperus cedrus</i> Webb and Berth.....	SPI 57080.....	50	2	(³)
<i>Juniperus hibernica</i> Gord.....	Nursery.....	35	4	(³)
<i>Juniperus phoenicea</i> L.....	SPI 65020.....	20	6	5-15
<i>Juniperus procera</i> Hochst.....	SPI 60553.....	30	3	5-10
Do.....	SPI 27505.....	50	0	0
<i>Juniperus virginiana</i> L.....	Nursery.....	35	15	5-20

¹ By "seedlings" is meant plants grown from seed at the University of California Citrus Experiment Station. "S" denotes the accession number of plants from the Rancho Santa Ana Botanic Garden, and "SPI", the plant introduction number of plants from the U. S. Department of Agriculture. "C. E. S." indicates large trees growing on the campus of the Citrus Experiment Station, and "Nursery" indicates trees purchased from a local nursery.

² Results observed 6 months to 1 year after inoculation.

³ Knobs.

Control punctures were made on the various hosts inoculated, but because of the limited plant material available, these controls were not numerous.

GALLS ON CUPRESSUS

The results of inoculations on species of *Cupressus* are listed in table 1. Galls (fig. 1) developed on all species tested, with the exception of *guadalupensis*.

The results on *glabra* and *montana*, however, were somewhat inconclusive. On small trees of *glabra* (*C. arizonica* var. *bonita*) the inoculations caused the development of small knoblike projections

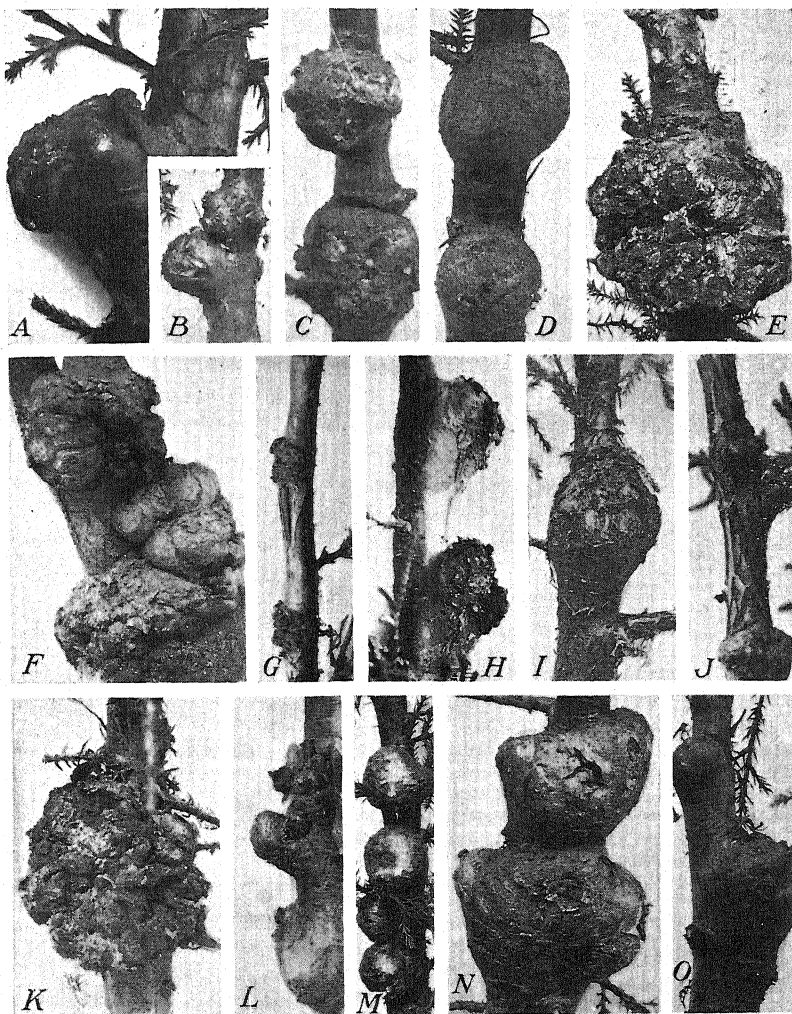


FIGURE 1.—Artificial galls produced on species of *Cupressus* by inoculation with *Bacterium tumefaciens* isolated from *Salix* sp. for all galls except *G* and *H*, for which cultures were isolated from *Prunus persica*. The time, in months, from the date of inoculation to the date of the photograph is indicated in parentheses following the description of the species: *A*, *arizonica*, a seedling (24 months); *B*, *arizonica*, S 2357 (14 months); *C*, *macnabiana*, S 2154 (18 months); *D*, *sargentii*, S 2149 (19 months); *E*, *macrocarpa*, a seedling (26 months); *F*, *benthamii*, SPI 114036 (12 months); *G*, *lusitanica*, SPI 73844 (8.5 months); *H*, same galls (24 months); *I*, *thurifera*, S 2356 (8 months); *J*, *torulosa*, SPI 112085 (5.5 months); *K*, *goeniana*, S 2181 (26 months); *L*, *forbesii*, S 2335 (26 months); *M*, *pygmaea*, S 2137 (14 months); *N*, *bakeri*, S 2131 (14 months); *O*, *duttoni*, S 2157 (8.5 months).

that failed to become typical galls (fig. 2, *F*). On mature trees of *glabra* growing on the campus, 50 series of 3 to 5 puncture inoculations each, were all negative. (*Guadalupensis* and *glabra* are the species of *Cupressus* frequently grown as ornamentals in southern California.) The galls on *montana*, 4 months after inoculation, were small knoblike growths 1 to 3 mm. in diameter that had grown from healed-over tissue (fig. 2, *D*, *E*). Although later observations indicated that these galls increased somewhat in size, their slow development suggests that the species has strong resistance. A few typical galls formed on *forbesii*, but most of the inoculations were negative in effect.

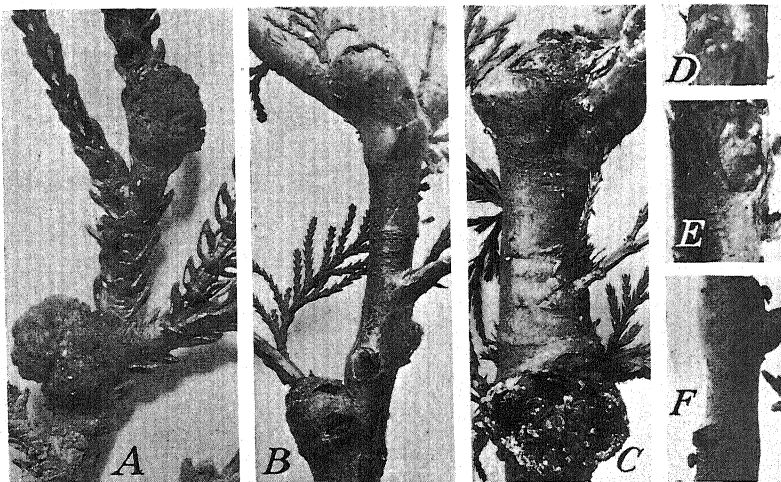


FIGURE 2.—Artificial galls produced on various species of Cupressaceae. Inoculations of A, D, E, and F were by cultures isolated from *Salix* sp.; inoculations of B were by cultures from *Prunus persica*, and inoculations of C were by cultures from *Libocedrus decurrens*. The time, in months, from the date of inoculation to the date of the photograph is indicated in parentheses following the description of the species: A, *Thujaopsis dolabrata* (9 months); B, *Chamaecyparis lawsoniana* showing two overgrowths with small projections on their upper parts and between these overgrowths a small knoblike development (11 months); C, *Libocedrus decurrens* showing two galls, the upper gall smooth and the lower gall rough (24 months); D, E, *Cupressus montana*, S 2067, small knoblike galls (4 months); F, *C. glabra*, knoblike projections (4 months).

The aerial gall on *Cupressus* may first appear as a globose swelling having a smooth bark from which small projecting knobs are often developed. This bark covering may remain smooth for about a year, but often the pressure of growth causes it to crack, and gum forms. The gall eventually becomes rough and resembles crown gall as it forms on other susceptible hosts. The artificial galls that develop below the soil are usually softer than aerial galls and have a smoother bark. The most susceptible species of *Cupressus* are probably *macrocarpa*, *pygmaea*, *thurifera*, *sargentii*, *lusitanica*, *duttoni*, and *arizonica*; but too few inoculations were made to permit definite conclusions to be drawn.

GALLS ON JUNIPERUS

The galls produced from inoculations on species of *Juniperus* are listed in table 1. The number of species available for testing was some-

what limited. Growths resulting from inoculations on *hibernica* were small papillalike protuberances that failed to develop into typical galls but were probably definite responses to the stimulus of the inoculation organism. The results of these inoculations were so inconclusive, however, as best to be regarded as negative. The inoculations on *cedrus*, SPI 57080, at first showed small overgrowths 2 to 3 mm. in diameter (fig. 3, *F*) that were regarded as gall initials; but these gall-like structures soon ceased to increase in size and, later, almost disappeared. Galls were readily formed on *virginiana* and *procera*, SPI 60553. Results on another tree of *procera*, SPI 27505,

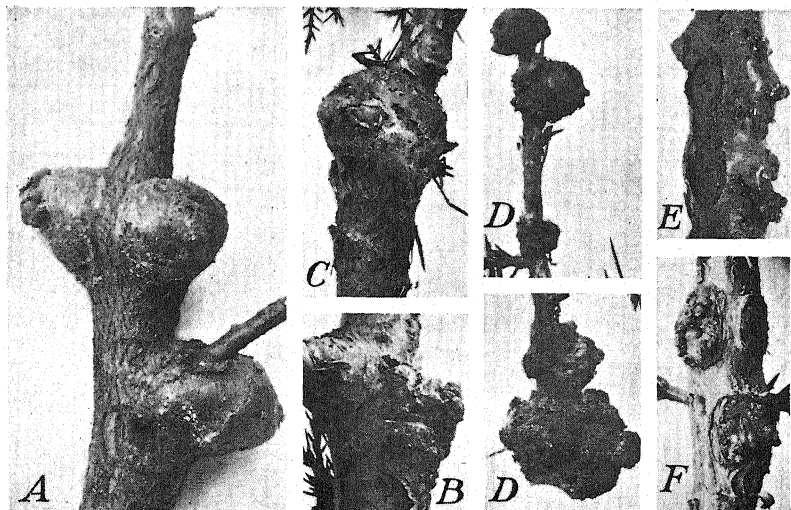


FIGURE 3.—Artificial galls produced on species of *Juniperus*. Inoculations of *A*, *B*, and *D* were by crown gall cultures from *Salix* sp.; inoculations of *C*, *E*, and *F* were by cultures isolated from *Prunus persica*. The time, in months, from the date of inoculation to the date of the photograph is indicated in parentheses following the description of the species: *A*, *procera*, SPI 60553 (14.5 months); *B*, *procera*, SPI 60553 (9 months); *C*, *virginiana*, nursery (15 months); *D*, *virginiana*, nursery (10 months); *E*, *hibernica*, nursery (18 months); *F*, *cedrus*, SPI 57080 (12 months).

were negative after 50 inoculations. Negative, also, were inoculations on *californica*. From the results of these inoculations it appears that *Juniperus* spp. are less susceptible than *Cupressus* spp. to inoculation with *Bacterium* (*Phytophthora*) *tumefaciens*.

GALLS ON LIBOCEDRUS

Nursery seedlings of *Libocedrus decurrens* were tested, and this host proved extremely susceptible to artificial inoculation. Galls frequently developed with a smooth bark, as shown in the upper gall of figure 2, *C*; but after about a year these smooth galls usually ruptured from growth pressure and became like galls on other susceptible hosts. The formation of galls on *L. decurrens* from artificial inoculations in earlier tests has already been described (5).

GALLS ON THUJA AND THUJOPSIS

Galls produced by inoculations on species of *Thuja* are listed in table 1 and shown in figure 4. The three species, *plicata*, *occidentalis*, and *orientalis*, responded readily to artificial inoculation and were apparently among the most susceptible species of the Cupressaceae. The galls were rough almost from the first.

Thujaopsis dolabrata was more difficult to infect than were the species of *Thuja*. Forty inoculations on a plant 2 feet high gave negative results. Rooted cuttings from this plant, however, proved susceptible and developed typical rough galls (fig. 2, A).

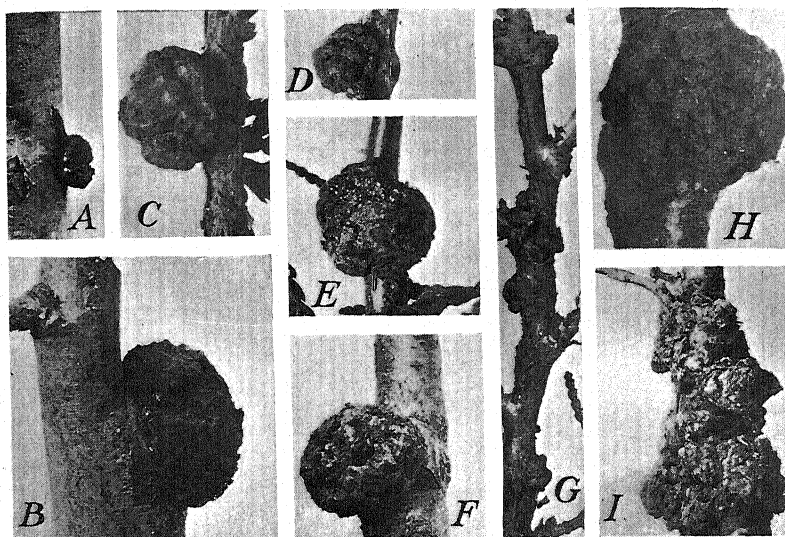


FIGURE 4.—Artificial galls produced on nursery trees of species of *Thuja*. Inoculations of A to H were by cultures isolated from *Prunus persica*; inoculations of I were by cultures from *Salix* sp. The time, in months, from the date of inoculation to the date of the photograph is indicated in parentheses following the description of the species: A, *Thuja plicata* (14 months); B, same gall (17 months); C, *plicata* (14 months); D, *occidentalis* (16 months); E, *occidentalis* (15 months); F, same gall (19 months); G, *orientalis* (10 months); H, *orientalis* (24 months); I, *orientalis* (24 months).

GALLS ON CHAMAECYPARIS

The Lawson cypress (*Chamaecyparis lawsoniana*) was the only species of *Chamaecyparis* tested. Ninety inoculations were made on three host trees, and the results of all except three were negative. Of these three inoculations, which were inconclusive, two showed overgrowths having smooth surfaces with small projections on their upper parts (fig. 2, B). At the time of the writing of this report, 4 years after inoculation, no abnormal increase in the size of these overgrowths has taken place, and they are less gall-like in appearance. Between the upper and lower overgrowths (fig. 2, B) can be seen a knoblike development which may have increased slightly in size; it is now 5 mm. in diameter and extends 6 mm. from the branch. The susceptibility of this species to artificial inoculation with the crown gall organism is yet to be demonstrated.

SUMMARY

This paper reports the results of artificial inoculations made with *Bacterium tumefaciens* Smith and Townsend on species of Cupressaceae. Cultures isolated from *Prunus persica* produced galls on *Cupressus*, *Juniperus*, and *Thuja*. Cultures from *Salix* sp. produced galls on *Cupressus*, *Thujopsis*, *Juniperus*, and *Thuja*. Cultures from *Libocedrus decurrens* gave negative results on all species except *L. decurrens*.

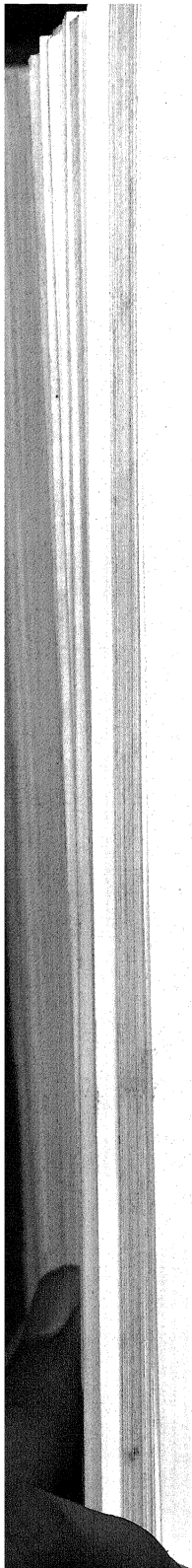
Sixteen species of *Cupressus* proved susceptible to inoculation. On *glabra* and *montana* occasional knoblike growths were formed, but no typical galls developed. One species of *Cupressus* (*guadalupensis*) was apparently resistant, as the results of all inoculations were negative. Inoculations on species of *Juniperus* produced galls on *virginiana* on *phoenicea* and on one plant of *procera*, but only small knoblike growths on *hibernica*, and *cedrus*. *Libocedrus decurrens* and the three species of *Thuja* tested proved susceptible and responded readily to inoculation. The original plant of *Thujopsis dolabrata* was negative in response, but rooted cuttings from this plant developed typical rough galls. *Chamaecyparis lawsoniana* developed nontypical overgrowths.

Control punctures were made on the various hosts inoculated. All of these injuries healed over in a normal manner.

The results of inoculations on species of Cupressaceae suggest the relative resistance of the various species but are not conclusive because of the differences in the environmental conditions of the plants tested and the small number of inoculations on some of the species.

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